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HORACE MANN WOOLMAN

PHYTOPATHOLOGY

VOLUME 23 NUMBER 12

DECEMBER, 1933

HORACE MANN WOOLMAN

1853–1932

HARRY B. HUMPHREY

Horace Mann Woolman was born in Damascus, Ohio, on December 23, 1853, and quietly passed from this life on December 23, 1932, in Orlando, Florida, at the age of 79 years. He had but a little while before gone to Orlando to escape the exactions of a northern Minnesota winter, and, as on similar occasions, was enjoying the kindly climate and peaceful atmosphere of that far-southern city, when, on the 16th of December, he was stricken down by paralysis from which he did not recover.

Mr. Woolman's forbears came to America with William Penn toward the close of the 17th century and settled in what was then known as Jersey. He was a direct descendant of a brother of John Woolman, the author of that early American philosophical classic, *The Journal of John Woolman*, and was, himself, a man endowed with the instincts of the student and scholar. True to the traditions of his Quaker lineage and background, Mr. Woolman was wholly and genuinely devoted to the philosophy of service, not for self but for the advancement and benefit of others.

Born at a time when, in the Middle West, facilities for acquiring a college education were extremely limited, it was not Mr. Woolman's privilege to enjoy such advantages until fairly late in life. Fired with an ambition to partake of the life intellectual, he availed himself of such educational facilities as there were in his boyhood environment and mingled with older men whose interests took them and their disciple afield into the realm of the "good life." Natural-born student that he was, he in time acquired a sufficient knowledge of civil engineering to qualify in that branch of applied science and he served in that capacity for several years. He it was who, while employed by the Chicago, Milwaukee, and St. Paul Railroad Company in the extension of its overland route through the mountains of Northern Idaho, surveyed one of the most difficult of the Coeur d'Alêne sections, and his recommendations were adopted. This was an accomplishment very much to his credit and one might, quite justifiably, have thought that, having gone thus far in his career in civil engineering, he would continue in its pursuit. But, even then, at the age of 56, the lamp of his unquenchable desire for the gifts of higher learning, still burning bright with enthusiasm and well-directed interest, we find Mr. Woolman registering at the State

College of Washington, where he pursued courses in botany, chemistry, bacteriology, and related subjects. The thing that through all his maturer years had been stuff for dreams and an insatiable longing was at last within his grasp, and he made the most of the opportunity. Incidentally, and with his characteristic, quiet enthusiasm he undertook also the study of the French and German languages. The upshot of this effort was that within a few years he had acquired a sufficient reading knowledge of both to enable him to abstract accurately such contributions to the literature on plant pathology as pertained to those research problems that were of peculiar interest to him.

Mr. Woolman, lacking a few credit hours of completing the requirements for college graduation, was appointed as field assistant in the Division of Cereal Crops and Diseases in 1917, with headquarters at Pullman, Washington. In that capacity he served ably until 1924, when, at the age of 70 years, he retired. So genuine was his interest in his chosen profession that for several summers after his retirement he kept himself informed and in touch with cereal-smut research, both here and abroad.

Toward the close of his career as a plant pathologist, at Pullman, and later at Corvallis and Moro, Oregon, Mr. Woolman acquired a working knowledge of the technique of plant breeding and applied himself effectively to the problem of the control of bunt through breeding bunt-resistant wheats. He produced a number of such varieties while in the service of the Department of Agriculture. In cooperation with Mr. David E. Stephens, he early investigated the susceptibility to *Tilletia tritici* of the 455 varieties included in the Department's wheat classification nursery at Moro, Oregon. Along with the varieties in this nursery, Mr. Woolman had under observation for resistance several selections from the Crimean group, a number of which were developed and have since shown marked resistance to many physiologic forms of *T. tritici*. The most outstanding of the sorts derived from Mr. Woolman's effort in this direction are the varieties Oro and Rio. The former is now being grown commercially in Southern Idaho. Segregating seed of hybrid strains from the cross Turkey × Florence were sent to Mr. Woolman by Dr. E. F. Gaines, of Washington State College. From this seed he developed a white-kernel variety, identical with the now well-known bunt-resistant Ridit, except for kernel color. This selection has since proved valuable as a parent in more recent crosses.

Later, Mr. Woolman made a number of wheat crosses between the bunt-resistant varieties Hussar and Banner Berkeley, Fürst Hatzfeld and Martin Amber, Hussar and Hohenheim 77, Ridit and Hussar, Martin Amber and White Odessa, Turkey and Florence, and a number of others. From these several crosses have come a few hybrid selections that have proved excep-

tionally resistant to bunt (*Tilletia tritici*), selections that may be especially valuable as parent material in subsequent breeding experiments.

It would be difficult to determine wherein Mr. Woolman made his most important contribution to our knowledge of bunt and its control. Suffice it to say that his excellent work speaks for itself; and those who were most intimately in contact with him, those who realized his devotion to research and caught his enthusiasm, were conscious of the value of his good work. The worth of the man is adequately expressed in these words, excerpted from a letter from Mr. David E. Stephens to the writer, written January 24, 1933: "I was distressed to learn of the passing of Mr. Woolman. I think few of us realized at the time he was carrying on his bunt investigations how much careful work he did and how much of his time and thought he devoted to his work. Mr. Woolman, I know, did his work thoroughly and carefully, devoting all his energy and capabilities to the problems he was investigating."

Those of us who knew Mr. Woolman best remember him as a man singularly free from prejudice, endowed with the graces of good-will, kindliness, and scrupulous consideration for the rights of others. Wholly free from any ostentation, a keen judge of human values, honest and honorable, he could not but endear himself to those whose good fortune it was to know him.

Mr. Woolman was, for several years, a member of The American Phytopathological Society and the American Association for the Advancement of Science. During the ten years of his research activity he contributed, in part or alone, the following papers:

Bunt or stinking smut of wheat. Wash. Agr. Expt. Sta. Bul. 126. 1915.

The prevention of wheat smut. Proc. Ann. Conv. Wash. State Grain Growers, Shippers and Millers Assoc. **10**: 45–49. 1916.

The wheat bunt problem in Oregon. Oregon Agr. Expt. Sta. Bul. 188. 1922. (With D. E. Stephens).

Cytological studies on the infection of wheat seedlings by *Tilletia tritici* (Bjerk.) Wint. (Abst.) Phytopath. **13**: 36–37. 1923.

Summary of literature on bunt, or stinking smut, of wheat. U. S. Dept. Agr. Dept. Bul. 1210. 1924. (With Harry B. Humphrey).

Studies in the physiology and control of bunt, or stinking smut, of wheat. U. S. Dept. Agr. Dept. Bul. 1239. 1924. (With Harry B. Humphrey).

Relative resistance of wheat to bunt in the Pacific Coast States. U. S. Dept. Agr. Dept. Bul. 1299. 1925. (With W. H. Tisdale *et al.*).

Infection phenomena and host reactions caused by *Tilletia tritici* in susceptible and non-susceptible varieties of wheat. Phytopath. **20**: 637–652. 1930.

BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE.

STOMATAL INFECTION WITH THE VIRUS OF TYPICAL TOBACCO MOSAIC

B. M. Duggar and Burt Johnson

(Accepted for publication March 10, 1933)

Incidental to extended studies of the physiological properties of the virus of typical tobacco mosaic, which are being carried on in this laboratory, it has seemed important to determine more precisely some of the possible avenues by which the virus may enter the plant when no wounds are apparent. Entrance of the virus through fresh wounds on leaves and stems has long been demonstrated and accepted. Whether these wounds are mechanical and artificial, such as mere abrasions, needle pricks, scratches, or cuts, or, as in the case of many other virus diseases, are the minute injuries made by certain sucking insects while feeding, there is "direct" penetration. Relatively few experiments seem to have been made with the idea of determining other possible means of entrance than through wounds. Allard (1) says, "Tests have also shown that simply pouring the sap of mosaic plants liberally upon the leaves of healthy plants is oftentimes sufficient to communicate the disease." Holmes (3, 4) has conducted extensive studies on types of injury on tobacco plants in relation to amounts of infection by the virus of tobacco mosaic.

In view of the great diversity of opinion regarding the nature of the virus agency, it is not indeed inconceivable that evidence for direct diffusion through uncutinized tissues might be sought by some investigators. Some work by one of us on the diffusion relations of this agency, to be published elsewhere, would seem to indicate that such efforts could yield only fruitless results. It may also be suggested at this time that the precipitation of the virus agent by fat solvents argues, on *a priori* grounds, against cuticular penetration, wholly aside from probable size considerations.

The possible entrance of the virus through stomata, however, has received far too little attention, and it is the purpose of this paper to report experiments bearing upon this means of entrance. It is highly important to determine to what extent stomatal infection may occur and whether or not a possible diurnal movement of the stomata of tobacco may have any influence on the entrance of the virus through the stomata, thus resulting in a greater percentage of infection in plants sprayed at one time of day as contrasted with plants sprayed at another time of day. If the stomata of tobacco exhibit marked diurnal movements, then differences in percentages of infection from hour to hour might be traced and correlated with differences in the stomatal apertures from hour to hour.

As a result of numerous studies on the part of many investigators, it is generally understood that stomatal movements of many plants are normally though perhaps indirectly controlled to a large extent by environmental conditions, particularly by light and humidity. The reasons for considering these effects "indirect" need not be developed in this paper. Temperature also is important, probably as much so because of its relation to light and humidity as to any more direct effect upon the plant. In general, there is abundant evidence to indicate that, while sensitive to light and other conditions, all the stomata of all species so far studied do not necessarily react exactly alike nor to the same degree to a given set of conditions.

Diurnal movements have, however, been largely correlated with light. Loftfield (6), working with a number of economic plants, found that "light induces the opening of stomata after daybreak," but that definite specific differences prevail. Humidity and temperature also were noted to have a marked effect on stomatal movements. On the other hand, there is evidence that the actual opening and closing of the stomata are in large part dependent on the species of plant studied. Loftfield noted such individual differences and Lloyd (5) previously furnished similar evidence on this point. Although Gray and Peirce (2) conducted their observations during daylight only, they found that the stomata of barley, wheat, oats, and rye are very sensitive to light, that the stomata are open widest when the light is most intense, and that they close when shaded, but there are some differences between species in this regard.

METHODS

The work here reported was done at different seasons. The experiments made during the winter were in a greenhouse, free from injurious insects, while during the summer an "insect-proof" tent out-of-doors furnished satisfactory semicontrolled conditions. Tobacco plants of Wisconsin Havana 142, a single selected strain of the Havana Seed type of tobacco, were used in all this work. The plants were carefully grown and at the time of inoculation (spraying) were in the 5-leaf or 6-leaf stage and were apparently free of wounds. A mulch of chopped cork was spread over the surface of the soil around the bases of the plants. The purpose of this ¼-inch layer of cork was to attempt to prevent infection through the roots by any of the virus suspension that might drain from the plants. However, very little of the sprayed material reached the soil, and if any should reach it and cause infections through the roots, such infections should be practically a constant factor. In one spraying experiment cork was not placed around the bases of the plants. The percentage of infection, however, was exactly the same as for those plants the roots of which were protected by

a layer of cork, but otherwise treated the same. All spraying experiments were so arranged that spraying of the various lots of a series was made each hour, or each 2 hours, or each 4 hours, according to the experiment, and extended through a continuous 24-hour period. Of course, in all operations great care was taken to see that the plants were under "normal" conditions and were not wounded or unduly disturbed at any time.

The suspensions of virus used for inoculations were prepared, unless otherwise noted, according to the standard methods employed in this laboratory.[1] Accordingly, the full strength, natural juice of diseased tobacco plants was diluted with distilled water to give 1:10 (1/10) concentration, and at this dilution the virus is freed of coarse impurities by diatomaceous earth (Celite). Controls were invariably employed to test the virulence of the 1/10, Celite-treated, infectious juice, which was used in spraying the plants. Control plants were inoculated by the needle-prick and scratch method. One drop of the infectious juice was placed in each of 3 locations on the plant; (1) in a leaf axil, (2) on the young stem, and (3) on a young leaf, each of these drops being immediately worked or pricked into the axil and scratched into stem and leaf blade with a sterile needle.

The spraying was carried out as follows: A De Vilbiss No. 15 atomizer was used to apply the virus to leaf surfaces in the form of a very fine spray. Both the upper and lower surfaces of the leaves were sprayed until the suspension began to form in drops on the leaves and run down the veins. The lower surfaces were sprayed by gently lifting the leaf with the nozzle of the atomizer and spraying while the leaf was so held. In this manner about 25 cc. of 1:10 Celite-treated, infectious juice were used on each set of 10 plants. A slight modification of this process for some of the experiments will be noted later. As soon as the spraying was completed, readings on temperature and humidity were taken, using for the latter a sling psychrometer. Also, at the same time, a strip of the upper epidermis from a single leaf, one from each of 2 plants (not used for inoculations), was removed and both strips were immediately plunged into a vial of absolute alcohol, these samples being taken for the purpose of determining the condition of the stomata in respect to size of stomatal opening. Using the same two plants, but different leaves, strips of the lower epidermis were removed and placed in a separate vial of absolute alcohol. This whole process (spraying, taking data on temperature and humidity, and removing strips of epidermis) on the average required 3 to 6 minutes, mostly about 5 minutes.

In order to carry out the above-mentioned procedures at night and still use as little light as possible, the following methods were used. The psy-

[1] Duggar, B. M. Proc. Soc. Exp. Biol. Med. 30: 1104–1109. 1933.

chrometer readings were started while the lights were still out. Then a small frosted bulb at one side of the greenhouse was turned on, and the readings on temperature and humidity were taken, and also the spraying of the 10 plants for that hour was done by this weak light. The small light was then turned out. The samples of epidermis were taken from plants distant from the weak source of light, so that even this brief illumination would have little effect on the plants from which the epidermis was taken. Over these plants was placed a large frosted bulb and the light from this bulb was used only for 30 to 45 seconds while securing the specimens of epidermis. For night work in the insect-proof tent, an ordinary oil lantern furnished light for all operations.

The samples of epidermis were stored in the alcohol until examined, when a saturated solution of Congo Red in absolute alcohol was substituted for the absolute alcohol used as the killing agent. The stained strips of epidermis were, when prepared for final study, mounted in absolute alcohol and examined under a microscope provided with an eyepiece micrometer, so that measurements of pore widths might be made. Since the idea was to get a general picture of the condition of the stomata of the tobacco leaf under the conditions of these experiments, each sample was studied carefully under high-dry magnification, observing a large but not fixed number of stomata—often as many as several hundred, varying with the size of the sample. By careful estimation and by checking measurements from time to time, the stomata were divided into 5 groups as follows: (1) pores entirely closed, (2) pores open to 2.5 μ across, (3) pores 2.5–5 μ across, (4) pores 5–7.5 μ across, and (5) pores more than 7.5 μ across.

The procedure of stomatal study used in these experiments follows quite closely that of Lloyd (5) and of Loftfield (6) and has been discussed in full by them. The method for the determination of stomatal size is open to criticism on a strictly quantitative basis. However, the purpose of this work was not to get absolutely accurate information about the stomata as such, but rather to secure information that might disclose any possible correlation between (a) size and movements of stomatal pores and (b) incidence of infection resulting from the spraying. For such a determination, all that is necessary is to secure a relative, quantitative expression of the characteristics of the stomata. By no other method, perhaps, could such a large mass of material be examined and such a general quantitative idea of the tobacco stomata be obtained. Besides, all conditions of the cultures, including environment and variety of host selected, are never exactly reproducible, in the hands of different investigators, so that it would be rather futile to set down absolute measurements.

In reference to the spraying technique, it could be objected that the mere impact of the spray particles might break the epidermal hairs, per-

mitting the entrance of the virus through wounds. However, even a casual examination of the resistance of these hairs would be against this interpretation; furthermore, we have been unable to find any evidence of injury from the use of a De Vilbiss atomizer. It should be noted, too, that no infestation of insects nor occurrence of wounds made by insects was observable on the experimental plants. That some slight wounds may occur on plants grown with the greatest care is granted, but the rôle of such wounds in infection requires demonstration, and experiments will be described later in this paper bearing upon this point. The possibility of root infection is remote and will also be considered in a later paper.

EXPERIMENTAL WORK

Preliminary work had indicated that under the conditions prevailing in the greenhouse used, infections could be obtained to the extent of 80 per cent of the plants sprayed, using the typical tobacco mosaic virus. Duggar (unpublished data), while working at the Missouri Botanical Garden, had secured a considerably lower percentage of infection by spraying full-strength, infectious juice (previously filtered through a porous porcelain filter) on healthy plants. He obtained under the conditions of those experiments 30 to 50 per cent of infections but was not satisfied with the technique employed nor with the control of the experiments.

Experiment I was a series of samplings of epidermis and of inoculations by spraying under reasonably controlled and recorded greenhouse conditions. The technique was essentially as outlined and discussed above. The experiment was started on April 2 at 4 p. m. and closed at 4 p. m. on April 3. Both days were bright and clear. Ten new plants were sprayed each hour throughout the 24-hour period. The control inoculations were made as explained above. Control *X* was inoculated at the beginning of the experiment—4 p. m., April 2. Control *Y* was made in exactly the same way and from the same lot of material, except that the plants were inoculated at the close of the experiment—4 p. m., April 3. The controls here were intended to test the virulence of the suspension of virus used in the whole series. In both cases the incidence of disease in the controls was 100 per cent.

Considering for the moment only the disease frequencies resulting from the 24 lots of sprayings, it will be seen (fig. 1, A) that the infections from this method of applying the inoculum are relatively high, averaging approximately 70 per cent; the range is 30 to 90 per cent, and in only 2 periods is it less than 50 per cent. It is equally important to note that there seems to be no discernible correlation between time of day or environmental conditions and the percentage of disease. The 2 low points in the infection curve, which appear in the daylight intervals, may be regarded for the

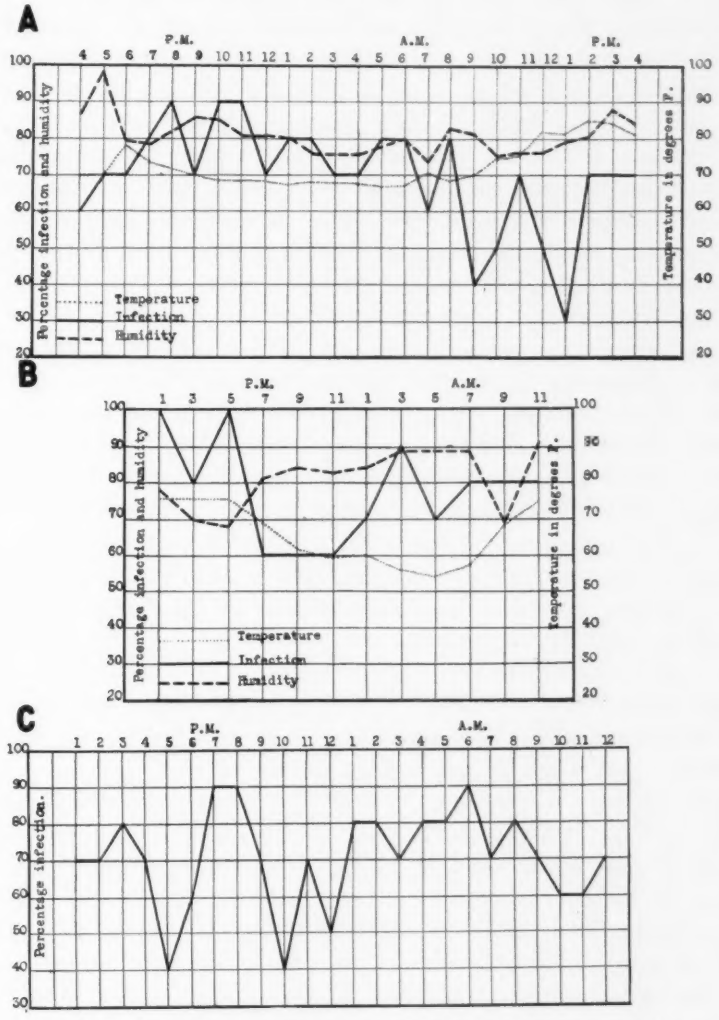

FIG. 1. Physical conditions at the time of and results of spraying tobacco plants with the virus of typical tobacco mosaic at intervals during 24-hour periods on different dates. A. Hourly in a greenhouse on April 2 and 3. B. At each 2-hour interval in an "insect-proof" tent on August 3 and 4. C. Hourly in a greenhouse on October 4 and 5. Apparently plants were not mechanically injured during the spraying.

moment as accidental. The relation of the various intervals to stomatal opening will be considered later.

Experiment II was carried out in the shade tent[2] on August 3 and 4.

[2] The plants were potted in the ordinary manner in the greenhouse and were allowed to remain there until well established, then they were transferred to an insect-proof tent and were allowed to grow there until they had reached the size generally used in this laboratory for inoculation, namely, the 5-leaf and 6-leaf stages.

This experiment was conducted exactly as was the one in the greenhouse, with these 2 exceptions: (1) Inoculations were made every 2 hours during a 24-hour period instead of every hour. (2) Night work was done by lantern instead of by electric light. The weather was partly cloudy. Although growing conditions in general differed considerably from those in the greenhouse, the temperature in the tent at the time of these sprayings was much the same as for the previous experiment, and the humidity in the tent was lower during about half the time. (Fig. 1, B.) Nevertheless, the curve of infection percentage is apparently as unrelated to environmental conditions as in the preceding experiment. Moreover, in the present experiment the 3 low points in the curve fall in the night interval. The controls again exhibit an incidence of 100 per cent diseased.

The data on the sizes of stomatal apertures in the 2 experiments are extensive. Since these data are somewhat simplified by the 2-hour intervals of experiment II, they will be given in detail for this series (Table 1). It will be noticed that there is a surprising variability in the extent of stomatal openings, and at no period of either day or night is there complete opening or complete closure of the stomata. Certainly, one would be disposed to draw the general conclusion that there are no striking correlations between width of stomatal openings of the tobacco thus grown and such environmental factors as temperature, humidity, and light, nor between width of stomatal openings and percentage of infection. However, a closer analysis of this last relation will be made, although it is fully realized that many other factors may affect the percentage of infection.

Assuming that infection may be related to extent of stomatal openings, the only possible method of analysis would seem to be that of summing the percentages of stomatal openings at any one time-period in each of the 4 size-classes separately (omitting, of course, the "closed" size-class), and then weighting these classes by multiplying by the proper factor, according to the areas involved in each class. Since the figures given in each line of the table represent 4 samples, 2 of the upper surface, and 2 of the lower surface, an average of each class may be taken, being less cumbersome. Then, a summation of the results thus weighted will give a figure that may be called the "infection probability coefficient."

The 4 classes referred to are (a) up to 2.5 μ, (b) 2.5–5.0 μ, (c) 5.0–7.5 μ, and (d) above 7.5 μ, in width, and for these classes it has been found that for all practical purposes it is satisfactory to weight these, respectively, as 1, 2, 3, and 4. This is based on the consideration that the stomatal pores are ellipses, and the area of an ellipse = πab, where $a = \frac{1}{2}$ the short diameter and $b = \frac{1}{2}$ the long diameter.

On this basis the coefficients from table 1 are found to range from 271 to 77.5, the averages being as follows: 100 per cent infection = 258; 90–70

TABLE 1.—*Data showing the percentages of stomatal pores falling into different-size groups, the temperature in degrees F., the relative humidity, and the percentages of infection due to spraying with the virus of typical tobacco mosaic at each two-hour period during a day. (Ten plants sprayed each period with 1:10 Celite-treated infectious juice.) Experiment arranged in an "insect-proof" tent, sprayings on August 3 and 4*

Time when stomata were open	Percentage of stomatal pores falling in the different-size groups																				Temp. °F.	Humidity %	Infection %
	Upper surfaces of leaves										Lower surfaces of leaves												
	Leaf No. I					Leaf No. II					Leaf No. I					Leaf No. II							
	Figures in microns					Figures in microns					Figures in microns					Figures in microns							
	0	To 2.5	2.5 to 5.0	5.0 to 7.5	> 7.5	0	To 2.5	2.5 to 5.0	5.0 to 7.5	> 7.5	0	To 2.5	2.5 to 5.0	5.0 to 7.5	> 7.5	0	To 2.5	2.5 to 5.0	5.0 to 7.5	> 7.5			
1 p. m.	0	0	20	80	0	...	...	...	...	...[a]	0	0	0	20	80	0	50	50	0	0	76	78	100
3 p. m.	0	40	30	30	0	5	35	50	10	0	0	0	25	60	15	0	0	15	60	25	76	70	80
5 p. m.	...	...	...	...	...	...	...	...	...	...[a]	0	10	10	30	50	0	45	40	15	0	76	68	100
7 p. m.	10	50	40	0	0	30	70	0	0	0	40	60	0	0	0	50	50	0	0	0	69	81	60
9 p. m.	10	70	20	0	0	80	20	0	0	0	0	15	25	40	20	60	30	10	0	0	62	84	60
11 p. m.	20	80	0	0	0	20	60	15	5	0	0	50	50	0	0	0	40	60	0	0	59	83	60
1 a. m.	50	25	25	0	0	30	50	20	0	0	0	5	10	35	50	5	90	5	0	0	60	84	70
3 a. m.	0	50	35	15	0	20	80	0	0	0	0	50	30	20	0	0	45	50	5	0	56	88	90
5 a. m.	40	40	20	0	0	35	60	5	0	0	40	55	5	0	0	40	55	5	0	0	54	88	70
7 a. m.	0	50	50	0	0	0	30	40	30	0	0	15	25	50	10	5	15	50	15	15	57	88	80
9 a. m.	10	40	50	0	0	0	50	50	0	0	0	10	50	40	0	15	20	55	10	0	69	68	80
11 a. m.	5	90	5	0	0	10	90	0	0	0	0	20	80	0	0	0	50	50	0	0	75	91	80
Average incidence of disease in per cent																							77.5
Control *X* (1 p. m.)																							100
Control *Y* (11 a. m.)																							100

[a] No readings made.

per cent, 155.9; 70–60 per cent, 115.3; 60 per cent, 104.2. These figures are at least suggestive of a relation between stomatal openings and percentage infection. Unfortunately, the greenhouse series is not so consistent. Nevertheless, even in that series, it is true that the higher percentages of infection have high coefficients, but not all of the lower percentages of infection exhibit correspondingly low coefficients.

In order to determine the influence of a more prolonged period of darkness on stomatal behavior in tobacco and on incidence of infection, 10 plants were placed in a dark room for 48 hours and then sprayed with 1/10, Celite-treated, infectious juice, and, after spraying, were allowed to remain an additional 12 hours in the dark room. While the plants were in the dark room they were well watered. Of these plants 60 per cent became diseased. At the end of the 48-hour period samples of epidermis were taken and later examined as previously described. Examination of the stomata showed percentages falling in the several size groups, as exhibited in table 2. Thus, it

TABLE 2.—*Percentage of stomatal pores falling into different size classes, in tobacco, after the plants were held for 48 hours in a dark room*

Leaf surface	Leaf No. I			Leaf No. II		
	Open to 2.5 μ	2.5 μ to 5 μ	5 μ to 7.5 μ	Open to 2.5 μ	2.5 μ to 5 μ	5 μ to 7.5 μ
Upper	50	50	0	50	50	0
Lower	40	40	20	10	40	50

is seen that in even prolonged absence from light, the stomata are at least partially open.

It should be noted that final observations to determine the amount of infection were made in all cases 3 weeks after spraying, since, in our experience, tobacco plants grown under ordinary conditions will express symptoms of mosaic, if present, well within this period in the usual infection experiments.

Owing to the possibility that the method of inoculation had resulted in wounding the plants and that the infections had occurred through such possible wounds, other spraying experiments were arranged in the hope of evaluating this possibility. It might conceivably be possible that lifting the leaves of the plants with the nozzle of the atomizer, in order to spray the under surfaces of the leaves, might cause injuries, and, if so, a mechanical wound inoculation of the plant might be made instead of an intended spray inoculation.

Experiment III, therefore, deviated from the practice for spraying as outlined under the heading "Methods," in that only the tops of the leaves

were sprayed. At no time were the leaves touched by the operator or by the atomizer. No cork was placed around the bases of the plants in this experiment. Nevertheless, there was no increase in the percentage of infection. This fact rather definitely indicates that the infections observed in these spraying experiments were not the result of virus penetration from the soil by means of the roots. Otherwise, essentially the same conditions prevailed in this experiment as in experiment I. Figure 1, C, graphically indicates that the experiments are definitely of the order of constancy observed in those already described, although in this latter work even stricter precautions were observed in the prevention of injury.

In still another experiment, using approximately 100 plants (but younger) in each series, and spraying each 4 hours during a 24-hour period, results comparable to those seen in table 2 were obtained. In this experiment also, the plants were not touched by the operator nor by his instruments. It would seem then that when this virus is sprayed on plants, entrance through stomata must occur, since there has been a strict control of the injury possibility. Nevertheless, it is essential to determine the effect of injured epidermal structures on the incidence of infection.

In order to test the relation of injury and noninjury of the epidermal hairs to incidence of infection, experiments were performed under 5 different environmental conditions. These experiments and the results obtained are briefly presented in table 3. Further explanations of (a) the

TABLE 3.—*Results showing the percentage of infection caused by dropping the virus of typical tobacco mosaic on injured and uninjured leaf surfaces, using plants somewhat differently treated. Numbers are the percentages of infections based on the use of 10 plants in each test. "C.T.I.J." signifies "1: 10 Celite-treated infectious juice"*

State of the leaves inoculated	Treatment of experimental plants				
		In insect-proof tent cage			
	A. Potted plants + C.T.I.J.	B. Potted plants + C.T.I.J.	C. Wilted potted plants + C.T.I.J.	D. Potted plants + natural infectious juice	E. Older plants in natural soil + C.T.I.J.
(1) Upper surfaces intact	30	20	10	40	10
(2) Lower surfaces intact	10	0	0	50	0
(3) Upper surfaces injured	20	100	70	100	40
(4) Lower surfaces injured	60	70	60	90	40
(5) Control–needle-pricks	70	100	100	100	100

state of the leaves inoculated (designated by the numerals 1–5 in table 3) and of (b) the general environmental conditions, together with sources of inocula (designated by the letters A–E in table 3), are necessary and are given in the correspondingly numbered and lettered paragraphs below.

(1) Each plant inoculated received 3–5 drops of the inoculum dropped upon the upper surfaces of each of 2 leaves. The part of the leaf supporting the virus suspension was apparently intact and uninjured.

(2) Precisely the same as in (1), except that the lower surfaces of the leaves received the drops of inoculum.

(3) Precisely as in (1), except that before dropping the suspension of virus on the leaves the upper surface of each leaf "inoculated" was vigorously and thoroughly brushed over with a camel's-hair brush, this treatment resulting in apparent injury of epidermal hairs.

(4) The lower surface of each of 2 leaves of each plant in the test treated exactly as described in (3).

(5) The control plants (10 in number, as in each block above) were inoculated by our standard needle-prick method, that is, pricking or scratching into the tissues a few (3) drops of the virus suspension indicated.

The further notes on environmental conditions, not adequately described in table 3, are as follows:

A, greenhouse-grown, early spring. B, tent-grown, midsummer. C, tent-grown, midsummer; the plants were deprived of water for 42 hours prior to inoculation. After the suspension of virus had dried on the leaves, the plants were watered and soon appeared normally turgid. D, tent-grown, midsummer. The suspension of virus (1:10 natural infectious juice) was not treated with Celite in this experiment. E, tent-grown, midsummer. The plants were growing in the field soil over which the tent was built. As these plants (Group D) were quite old, they were cut back to yield new shoots, and they were inoculated one week after topping.

From the results in table 3, lines (1) and (2), it will be seen that some infections may result from dropping the infectious juice on the apparently uninjured surfaces of leaves. This method is, however, rather erratic and is doubtless dependent to some degree, possibly to a considerable degree, upon the "spread" of the drop on the leaf.[3] The amount of leaf covered is less, of course, than when a spray is employed, and the percentage of infection averages considerably less. As would be expected, the freshly injured leaf surfaces, table 3, lines (3) and (4), afford a high percentage

[3] In this connection it should be noted that in some earlier experiments in which drops of diluted infectious juice were placed on tobacco leaves the incidence of infection amounted only to an occasional diseased plant. One of these experiments was made in cloudy weather, the plants were exceptionally turgid, and the immediate spreading of the drops was insignificant.

of infections, the older plants exhibiting, in general, the lowest average. Another factor requiring consideration is that of the "age" of the wound. Our experiments confirm, in general, the findings of Holmes to the effect that the higher incidences of disease are obtained when the inoculating needle carries the virus with it, or when inoculation consists in pricking through a drop of the virus suspension. Commonly, placing drops of the virus on old wounds is far less effective than a similar treatment of fresh wounds. Returning then to a consideration of our sprayed plants, it may be deduced that with the precautions taken to avoid fresh wounds, and, wholly aside from the duplication of controlled features already referred to, the existence of old wounds would not seem to constitute an important factor in disease production.

Since it is clear that a high incidence of disease results from spraying healthy, young tobacco plants with infectious juice and since the probability seems to be established that stomatal penetration of the virus can well occur, some explanation of the whole phenomenon of infection should be available. There are then 2 aspects of this problem, (1) the entrance of the suspension into the substomatal cavities and (2) the mechanism of penetration into the cells. The first-mentioned is amenable to experimental study and will be considered first, although at this time our observations are merely preliminary. If a glass slide or Cellophane sheet is swung rapidly through a spray of greatly diluted India ink the spray droplets on hitting the target will be found to have taken the form of ellipsoidal films. We have measured these films but have not measured the droplets. We have estimated these droplets, on the basis of the character and small diameter of the films, to be approximately one-third the diameter of the latter, the smaller droplets probably 10 μ in diameter. It is possible that such droplets might be shot through the fully open stomata; but there seems to be a far greater likelihood that films formed as the smallest droplets strike the inner edges of the guard cells would be forced through the opening, just as the long-ellipsoidal films are produced on the moving glass or Cellophane.

In order to make an approximate test of the entrance of suspension droplets through the stomata, an alcoholic solution of nigrosin and an aqueous suspension of India ink were prepared and each was sprayed on a separate series of 10 plants each. After evaporation of the liquids, samples of the epidermis were secured and fixed in absolute alcohol. Microscopic examination of these samples showed, in the case of the India ink, that (a) large blotches of the ink adhered to the surfaces of the leaves and that (b) from 3 to 5 per cent of the stomata not covered by the blotches were wholly or partially filled by the ink. This observation was confirmed by a later test in which the presence of the India ink in some of the sub-

stomatal cavities was readily identified but no quantitative relations established. The nigrosin, of course, was dissolved by the fixing alcohol but, nevertheless, enough of the particles remained in a few of the stomata to suggest at least a considerable penetration of this material, as well.

In reference to the mechanism of penetration into the living cell, this problem is wholly hypothetical and will be considered more at length in a later paper. It may be said, however, that the senior writer has long assumed a dominant rôle of protoplasmic bridges, or plasmodesma, in the transfer of the virus from cell to cell. For the moment, then, the question may merely be raised as to whether or not interfacial tension may be adequate to insure the "creeping" of the virus particles between the walls of cells of the mesophyll to points where adsorption or picking up of the particles by the protoplasmic fibrils might take place.

It seems rather remarkable that up to the present we have found the incidence of infection very low when leaves are partially immersed in a suspension of virus. In one of the early experiments in which this was true the suspension was confined by a ring of white vaseline, the leaves treated being supported horizontally. It was determined to elaborate somewhat this type of experiment, and the series outlined below was arranged, yielding infection results as indicated in each case. Ten plants were used in each of the 7 experiments referred to, a vigorous, attached leaf in each case (except in the control) being immersed, by tilting, in the suspension described. (1) One leaf of each plant was largely immersed in a vessel of 1/100 C.T., infectious juice, remaining 3 weeks. Result: 1 plant diseased. (2) Same as in (1), except the tip of the leaf was excised with sterile scissors before immersion. Result: 1 plant diseased. (3) A healthy leaf of each plant was immersed, as in the preceding, and then the entire set-up placed in a partial vacuum, amounting to 11 cm. of mercury, for 10 minutes; then, after removal from the partial vacuum, the plant was returned to growing conditions, the leaf remaining partially immersed for 3 weeks. No water-soaked areas developed. Result: 1 plant diseased. (4) Same treatment as in (3), except that the leaf was removed from the virus suspension after the partial-vacuum treatment. Result: 2 plants diseased. (5) Same treatment as (3), except that prior to treatment in the partial vacuum the leaf tip was excised with sterile scissors. Result: 1 plant disease. (6) Treated as in (4), except that the tip of the leaf was excised before the partial-vacuum treatment. Result: 3 plants diseased. (7) Control, needle-prick inoculation, with the same 1/100 C.T.I.J. Result: 8 plants diseased.

From these results two suggestions may be made: First, dipping a leaf into a requisite volume of water is not comparable with spraying or other

similar procedure, and it seems necessary to infer that surface-tension relations are not the same.

On the other hand, the presence of any important "natural" wounds or of those induced in the plants of experiments (2), (5), and (6), described above, would seem to offer avenues of entrance, if such wounds were highly effective for virus penetration. The second suggestion is that the plants of these experiments required more "manipulation" than those sprayed with the virus suspension, and, since no significant percentage of disease results in these immersed plants, we may feel relatively certain that manipulation and corresponding injury did not constitute a significant factor in the incidence of disease obtained by spraying.

It should be pointed out that as a result of spraying the suspension on leaf surfaces there are formed relatively vast spreading films, as well as droplets. We do not yet know what the condition of the surface is when immersion occurs; possibly the stomata are blocked by air bubbles.

The various experiments and observations reported in this paper seem to establish with reasonable probability the view that stomatal infection may occur when a suspension of the virus of typical tobacco mosaic is atomized or sprayed upon the young leaves and shoots of the tobacco plant.

SUMMARY

In this paper there is presented a discussion of the technique of spraying tobacco leaves with a virus suspension in the effort to determine whether or not stomatal infection may occur. In respect to this infection problem a study has been made of stomatal behavior in the tobacco plant during 24-hour periods in 1-hour and 2-hour intervals in relation to such factors as light, temperature, and humidity. No significant correlation has been detected between the number of stomata open nor the extent of opening and these environmental factors. At no observation-time were all the stomata on a leaf closed.

The average incidence of infection from spraying is significantly higher than previous experiments had indicated. Cognizance has been taken of the possibilities of entrance of the virus by means other than through the stomata, but the evidence deduced is opposed to such possibilities. Some degree of correlation may be found between percentage of infection and the extent of stomatal opening, but no correlation is found between percentage infection and environmental factors within the range of the experimental conditions.

Dropping a virus suspension on regions of the leaf where epidermal hairs had been extensively injured by rubbing did not increase the amount of infection over that induced by spraying with the suspension. On the

other hand, placing large drops of virus suspension on uninjured leaves induced a lower percentage of infection.

A very low incidence of infection occurred when individual, vigorous, leaves attached to the plants were submerged for varying lengths of time in suspensions of the virus, and attention is directed to surface-tension effects.

It is suggested that the atomized particles of virus suspension may be directly "shot" into fully open stomata, but the probability is greater that the spreading of those films formed as the spray particles impinge on the walls of the open stomata is a chief means of entrance. A problem of much significance is the mechanism of penetration of the virus particle into the living cell from the substomatal cavity, since this may be related to the problem of virus movement from cell to cell.

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SEEDLING STEM BLIGHT OF FIELD BEANS CAUSED BY RHIZOCTONIA BATATICOLA AT HIGH TEMPERATURES[1]

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Following periods of unusually high temperatures, a destructive seedling disease of Red Mexican, Red Kidney, and Pink beans, *Phaseolus vulgaris* L., caused by *Rhizoctonia bataticola* (Taub.) Butler, was observed in 1929, 1931, and 1932 in the Sacramento and San Joaquin valleys of California, where beans for dry-bean production are grown during the dry hot summer months. Infection occurs near the cotyledonary node and usually kills the seedlings. The fungus was consistently isolated from the lesions and its pathogenicity to seedlings at high temperatures proved in inoculation tests.

The rather wide-spread occurrence of this fungus in California on maize, beans, and cowpeas in the mature stages of these crops has recently been reported by Mackie (5). The disease of beans caused by this fungus, or closely related forms, has been found in many parts of the world, particularly in tropical regions. Stem infection of jute and cotton seedlings by this fungus, then designated as *Rhizoctonia solani*, was found by Shaw (7) in India and in inoculation tests he obtained infection of jute, cotton, groundnut, and cowpea seedlings. He later (9) reported the fungus on *Phaseolus lunatus*. Briton-Jones (2) found the fungus causing a root rot of beans and cowpeas in Egypt and obtained infection of seedlings of both host species. Small (10) found the fungus on beans in Uganda, obtained infection of bean seedlings, and later (12) observed the organism on beans in Ceylon.

In the United States, the ashy stem blight of beans reported from South Carolina by Ludwig in 1923 and 1925,[3] from Georgia by Boyd[4] in 1927, and from Mississippi in 1926 by Wedgworth (17) is caused by *Macrophomina phaseoli* (Maubl.) Ashby, which the work of Shaw (8), Ashby (1), and Haigh (4) has shown to be a pycnidium-producing strain of *Rhizoctonia bataticola*. J. T. Barrett observed this disease of beans at Riverside, California, in 1919. This fungus has also been found on beans in Palestine by

[1] Contribution from the Division of Plant Pathology, Branch of the College of Agriculture, University of California, Davis, California.

[2] The writer wishes to acknowledge the helpful advice of Professor Ralph E. Smith and the assistance of Dr. Max W. Gardner in the preparation of the manuscript.

[3] A new stemrot of bean in South Carolina. U. S. Dept. Agr. Bur. Pl. Industry Pl. Dis. Rptr. 9: 60. 1925. (Mimeographed.)

[4] U. S. Dept. Agr. Bur. Pl. Industry Pl. Dis. Rptr. 10: 18. 1926. (Mimeographed.)

Reichert and Hellinger (6), who obtained infection of bean seedlings in inoculation tests.

SEEDLINGS KILLED AND STAND REDUCED

The damage caused by the disease is primarily due to irregularity of stand and subsequent reduction in yield. If environmental conditions favor its development, many young plants are killed as they emerge, or shortly after they emerge, from the soil. Diseased plants quickly dry up and are hidden by adjacent healthy plants or are covered with soil during cultivation. The grower in many cases does not recognize the trouble as a disease and attributes the poor stand to poor germination or to hot-weather injury. It is only in extreme cases, *i.e.*, where a high percentage of the plants are killed, that alarm is caused. There is sufficient evidence to indicate that in certain seasons, notably those in which high temperature prevails when the plants are in the seedling stage, serious damage to the bean crop in interior California has been caused by the disease.

Seedling stem blight was first observed by the writer on July 25, 1929, in a 40-acre field of young Red Mexican beans near Lodi, in the San Joaquin valley. The many irregularities in stand and presence of dead plants indicated that the disease had been present in the field for at least 2 weeks. Approximately 50 per cent of the plants were dead or dying and diseased plants occurred more or less uniformly throughout the field. An intensive survey of the bean plantings in the surrounding area revealed the presence of a similar disease in many fields where the plants were approximately of the same age as those in the field noted above. The severity of the disease varied from a trace to more than 10 per cent. Scattering cases of the blight were observed and many short skips or irregularities in stand indicated that the disease had been more severe earlier in the season. One could find the small, dried-up, diseased plants when the soil that had been piled up around the plants by the cultivator was removed. Careful inspections of the fields in which the blight occurred in July were made during the remainder of the season and the disease was not observed on plants that had escaped infection in the seedling stage. A survey of the large bean-producing area in the upper delta region immediately west of the Sacramento River failed to show the presence of the blight. No cases of the disease were found in the extensive Blackeye cowpea plantings of the San Joaquin Valley.

The blight appeared again in 1931 on seedling beans throughout the Sacramento Valley. In some fields, the stand was reduced from 10 to 20 per cent and certain areas in these fields showed a 50 per cent mortality of seedlings. In some plantings many of the young seedlings were killed before reaching the surface of the soil. It was again observed that the plants that had escaped the disease in the seedling stage did not become infected. It was not found in plantings of Henderson's Bush Lima beans.

On July 13, 1932, an examination of 600 acres of Red Kidney beans was made near Orland, in the upper Sacramento Valley, where blight was found to be severe over the entire planting; approximately 60 per cent of the young plants were dead or dying. The following day the disease was found to have reduced the stand from 30 to 40 per cent in a field of young beans near Lodi, in the upper San Joaquin Valley.

FIELD OUTBREAKS FOLLOW PERIODS OF HOT WEATHER

The sudden appearance of seedling bean blight in 1929 followed an extended period of high temperature. Practically every field where the disease occurred had been planted immediately before or during a period of high temperature extending from June 21 to July 5. The daily mean temperature recorded by the U. S. Weather Bureau at Stockton, not far from Lodi, was near or above 80° F. during the above named period (Fig. 1).

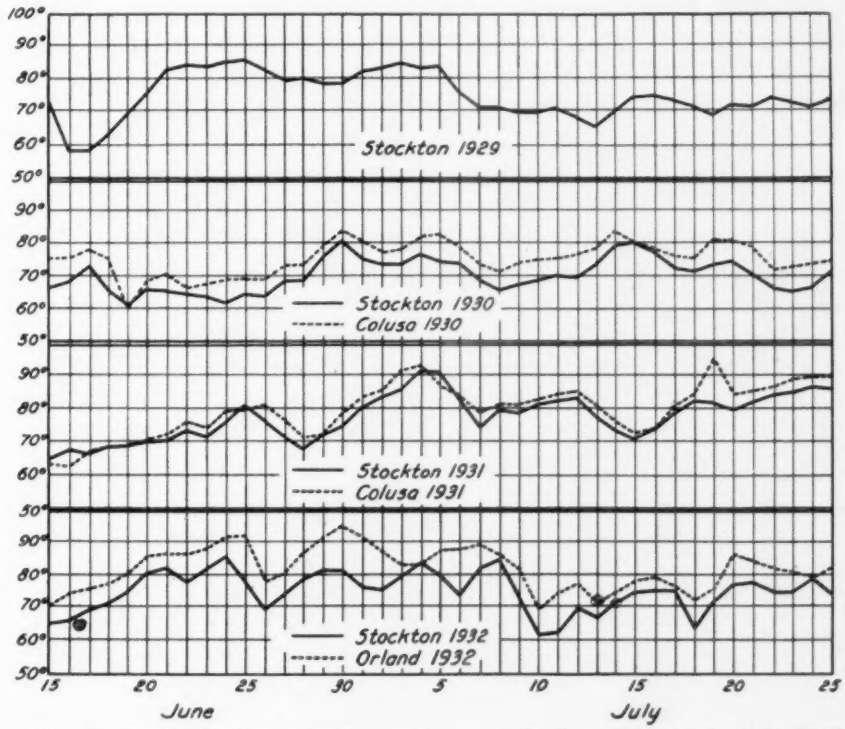

FIG. 1. Graph showing mean temperature records from June 15 to July 25 for Stockton, 1929 to 1932; Colusa, 1930 and 1931; and Orland, 1932. During this period most of the beans are planted in the valleys of central California where epiphytotics of seedling stem blight have been observed following prolonged periods of a mean temperature of around 80° F. or above. Outbreaks of the disease occurred from July 5 to July 15 near Stockton, 1929, 1931, and 1932; near Colusa, 1931; and near Orland, 1932. Only a few cases of the disease were found near Stockton and Colusa in 1930 when the mean temperature was below 80° F. for most of the period shown on the graph.

In fact, maximum temperatures of 95 to 106° F. were recorded for every day of this period.

During the summer of 1930, repeated observations in the region where the blight had occurred in 1929, as well as in other regions of the interior valleys, were made and only a very few cases of the disease were found. Reference to figure 1 will show that no prolonged period of high temperature prevailed at Stockton nor at Colusa, in the Sacramento Valley, and that mean temperatures as high as 80° F. or above were rarely attained.

In 1931, when the blight was prevalent near Lodi, again, and also near Colusa in the Sacramento Valley, it occurred almost entirely in fields that had been planted just prior to or during the early days of an extended period of high mean temperature from June 30 to July 13. (Fig. 1.) The mean temperature was 80° F. or above during most of this time, and the maximum temperature at Colusa ranged from 96° to 108° F., and at Stockton was 100° F. or above on 8 of the 14 days. Many fields that had been planted earlier, in which the plants had attained some size before the extremely hot weather, showed no evidence of blight. The disease was severest where the plants had emerged from the soil during the period of high temperature. On July 17, 1931, an examination of approximately 500 acres of young Red Kidney beans on 8 farms near Colusa showed a loss of 5 to 50 per cent from seedling stem blight. One large planting of over 300 acres was damaged 5 to 10 per cent, and a 20-acre field, where the plants were just coming up during the severest part of the hot period, showed a loss of over 50 per cent. Several earlier plantings of Red Kidney beans where the plants had developed trifoliate leaves before the hot period did not show evidence of the disease.

In 1932 seedling blight was found very severe on July 13 near Orland, following a period of high temperatures extending from June 20 to July 9 (Fig. 1) during which the mean daily temperature was well above 80° F. on all but one day, was above 90° F. on 5 days, and reached 95° F. on June 30. The maximum temperature ranged from 101° to 113° F. for 17 of the 20 days and was above 95° F. the other 3 days.

Thus the occurrence of blight in the field was correlated with periods of hot weather when the mean daily temperature was 80° F. or above. During the 4 years 1929 to 1932, 3 such periods occurred and it was only during these periods that the blight seriously damaged bean seedlings. It is not unusual in the central valleys of California for periods of 1 to 3 weeks of hot weather to occur in June and July. It has been noted that beans planted so that the plants had developed the first trifoliate leaves before such a heat wave escaped the disease. Also, plantings made after the middle of July have not been observed to suffer materially from seedling blight.

The geographical distribution of this fungus as a pathogen suggests

that it is favored by high temperatures. Small (10, 11) reported that the wilt of beans caused by this fungus occurred at high temperatures and Taubenhaus (16) states that its attack on sweet potatoes is most common in overheated houses maintained at 80° to 90° F. during the sweating process and in storage bins next to or nearest the stoves.

SPECIES AND VARIETIES SUSCEPTIBLE

Under field conditions, Red Mexican, Red Kidney, and Pink beans, *Phaseolus vulgaris* L., have been seriously attacked, while not much damage has been observed in fields of Henderson's Bush Lima beans, *P. lunatus* L. var. *lunonanus* Bailey, or Blackeye cowpeas, *Vigna sinensis* Endl. In the 600-acre planting of Red Kidney beans, which showed a 50 to 60 per cent depletion in stand because of this disease in 1932, a small acreage of Blackeye cowpeas planted at the same time in one of the large bean fields showed no cases of the disease. Mackie (5) found the late-season attack of the fungus in California on Blackeye cowpeas and all varieties of beans except the Large Lima and Hopi Lima.

In a small experimental plot of infested soil at Davis, planted at intervals during the summer of 1932 with a number of species and varieties of

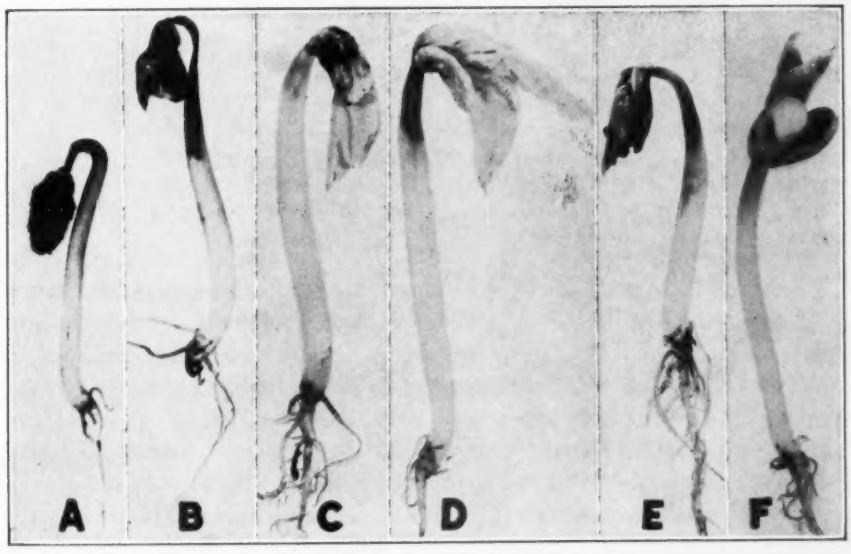

FIG. 2. Seedling Red Kidney beans showing different stages of stem blight caused by *R. bataticola* at high temperatures. A. Collapse of upper hypocotyl before the cotyledons reached the soil surface. B. Upper hypocotyl and plumule killed soon after emerging from the soil. C–E. Progressive stages in the development of stem blight on the hypocotyl and plumule after the plants have pushed through the soil. F. Healthy plant.

beans, light infection occurred on Scarlet Runner, *P. coccineus* L., Burpee's and Fordhook Bush Lima, *P. limensis* Macf. var. *limenanus* Bailey, and the following varieties of *P. vulgaris*: Speckled Cranberry, Davis White Wax, Kentucky Wonder, Golden Wax, Pink, Red Mexican, Dwarf Horticultural, Black Valentine, Refugee, Bountiful, and Stringless Greenpod.

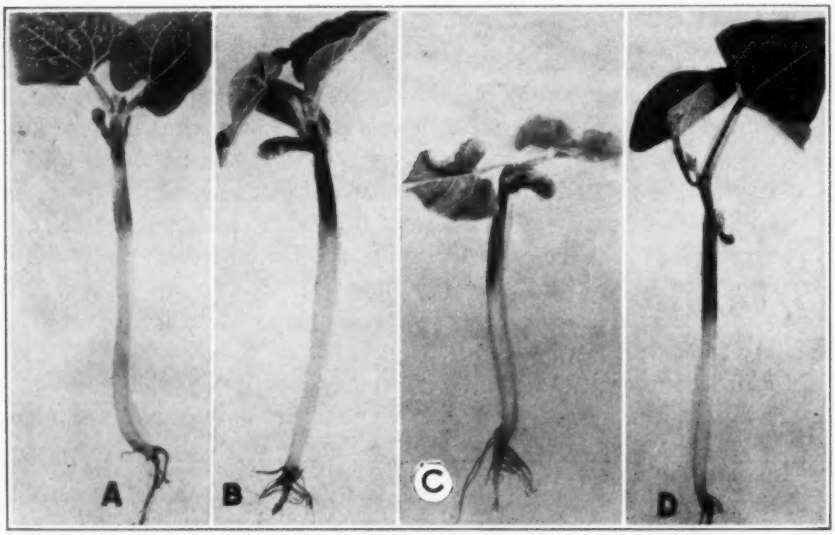

FIG. 3. Symptoms of stem blight on seedlings of Red Kidney bean after the primary leaves have developed. A. Incipient lesion at the cotyledonary node. B. Lesion approximately one day older than A. C. Linear lesion on one side of the hypocotyl and drooping of the primary leaves as a result of the invasion of the plumule by the fungus. D. Rapid invasion of the epicotyl and a primary leaf petiole, causing the petiole to collapse before the lamina wilts.

Seedlings Infected Near Cotyledonary Node. The fungus attacks the hypocotyl or epicotyl of the bean seedling either before or soon after it emerges from the soil, causing a conspicuous, black, sunken canker about the base of a cotyledon. (Fig. 3.) A somewhat similar type of hypocotyl infection was observed by Shaw (7) on jute, cotton, groundnut, and cowpea seedlings. Briton-Jones (2) obtained infection on cowpea and bean seedlings. Small (10) and Reichert and Hellinger (6) also obtained infection of bean seedlings. Small (13) found infection on seedlings of certain woody hosts, such as tea plants, and believed that infection occurred through the roots, though he (14) reports an instance of collar rot of a tea seedling. Butler (3) found that tea seedlings were invaded in the stem rather than through the roots.

The first evidence of the disease is the appearance of a small somewhat irregular, dark, sunken lesion on the stem at the base of a cotyledon petiole.

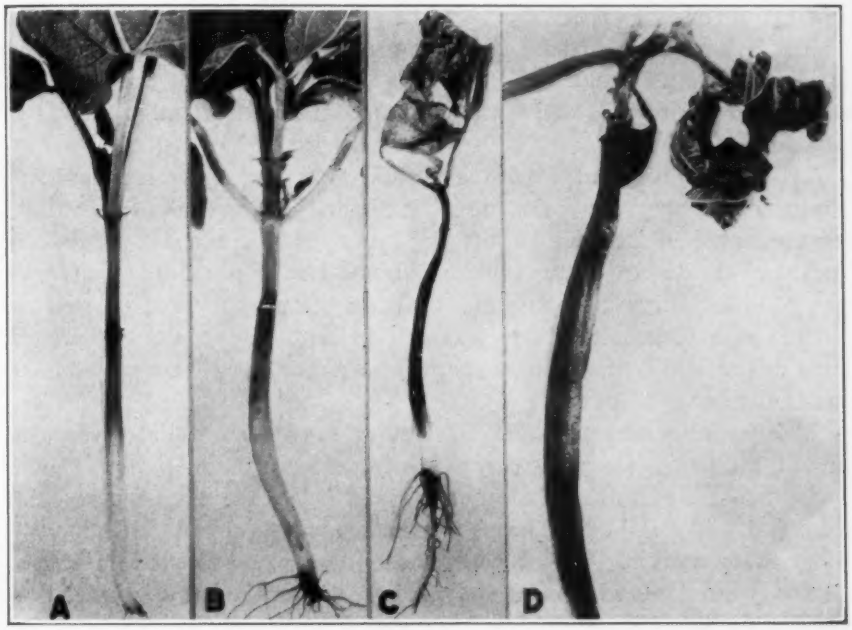

FIG. 4. A. Incipient lesion at cotyledon scar on a Pink bean plant where infection occurred after the trifoliate leaves started to develop. B. Late stage of the disease on an older Red Kidney bean plant showing the restricted development of the canker, concentric markings and a lateral break of the stem at the cotyledonary node. C. Advanced stage of stem blight of Red Kidney bean plant showing the death of the above-ground parts while the lower hypocotyl and roots are still apparently healthy. D. Red Mexican bean plant, killed by *R. bataticola*, showing small black, sclerotial bodies embedded in the cortical tissue.

(Fig. 3, A.) Infection spreads rapidly down the hypocotyl and up into the plumule, often involving the petiole of one of the primary leaves. (Fig. 3, D.) The progress of the lesion may be so rapid that the entire stem is involved, and the plumule is killed. (Figs. 2, A, B, and E and 4, C.) Often the petiole of a primary leaf is invaded so rapidly that the petiole tissues collapse before wilting of the lamina is evident. (Fig. 3, D.)

When very high temperatures prevail, the cotyledons and plumule are often invaded before the plant reaches the surface of the soil and when the U-shaped hypocotyl is pushed through the soil by the healthy roots below, the upper end of the hypocotyl, attached cotyledons, and plumule have been killed. (Fig. 2, A.) In other cases the plumule pushes out from between the cotyledons and starts to grow, but its development is quickly arrested by the progress of the fungus in the hypocotyl and epicotyl and the young plants are killed. (Fig. 2, B and E.) Many of these plants never reach the surface of the soil or, if they do break through the soil, they are in such

a weakened condition that they die and dry up in a few hours in the hot dry winds. Where irregularities in stand occur, it requires a rather careful examination of the surface of the soil, as well as just under the surface, to realize the extent of the damage to the young plants from this phase of the disease.

Often when infection is delayed until after the primary leaves are fully developed, the progress of the fungus is materially slower than when infection occurs before or soon after the plant emerges from the soil. The development of the disease on such plants is usually more pronounced along one side, resulting in the drooping and death of the primary leaf on that side and the yellowing of trifoliate leaves if present. Such plants are easily noted in the field by their stunted appearance and the yellow wilted condition of the leaves on one side.

If infection does not show until after the first trifoliate leaves are formed, the lesion is usually very much retarded in its development and is confined to the area immediately above and below the cotyledonary node. (Fig. 4, A and B.) Such lesions are sunken, dark-colored cankers with a rather sharp margin. It is not unusual to observe a concentric pattern in old slowly developing lesions. (Fig. 4, B.) Often the lesion extends down the stem to the ground line, but in no case has it been found extending to the roots. (Fig. 4, C.) However, even though the lesion is small, the stem becomes brittle at this point and usually is broken off sooner or later by wind or cultivation.

Sclerotial bodies are produced in the older parts of slowly developing lesions in 3 to 4 days, and in such lesions an abundance of small black bodies of this sort protrude from the cortex and are visible after 5 to 6 days. (Fig. 4, D.) Formation of sclerotial bodies is not so rapid on young plants infected while emerging from the soil, but after a few days these may be found in the dead tissues.

Macroscopic observation distinctly indicated that the tissue around the base of the small cotyledonary petiole was the point where the fungus had entered. Longitudinal and cross-sections, cut through the hypocotyl and attached cotyledon in both infected and healthy seedlings, failed to show any wound or natural opening in the tissues at the point of infection. Examination of young lesions showed that there was no invasion of the cotyledonary petiole until after the stem tissue at its base had been invaded. It, therefore, is assumed that the fungus is able to penetrate the unwounded epidermis, possibly through the area of primordial tissue in the axis of the cotyledon.

An examination of stained microtome sections through incipient lesions showed the fungus to be intercellular and confined in the early stages to the cortical tissues. The progress of the mycelium under favorable conditions

is rapid, causing a disintegration of the tissues. Growth of the mycelium is much more rapid longitudinally through the cortical tissues than radially or tangentially. Later the fungus penetrates the inner tissues and causes the stem to collapse.

RHIZOCTONIA BATATICOLA ISOLATED FROM DISEASED SEEDLINGS

Hundreds of isolations from diseased seedlings collected in the field and from artificially inoculated plants have consistently yielded pure cultures of *Rhizoctonia bataticola*. This fungus grows readily on potato-dextrose agar at ordinary laboratory temperatures. Mycelial growth is rapid, spreading, white, with slight aerial hyphae at first, but soon becomes more dense and prostrate. In 2 to 3 days at 25° to 30° C., the center of the mycelial mat begins to turn a greenish black, and at the same time, abundant, minute, black, round sclerotial bodies appear in the discolored area. Within 4 to 6 days the surface of an agar Petri dish usually is covered by the colony, and the small black sclerotial bodies, uniformly embedded in the upper strata of the medium, are so abundant that the fungus is black in appearance. In hundreds of agar plates and tube cultures examined, no pycnidia have been observed.

On sterilized young bean stems abundant growth occurs, and in a few days the small black sclerotial bodies appear in great abundance in the cortex. Such cultures have been examined from time to time over a period of more than one year during which they were allowed to dry thoroughly, but no pycnidia were found. Moreover, bean seeds were planted in sterilized moist sand in large test tubes with small bits of young agar cultures placed on the surface of the sand. The resulting seedling plants readily became infected, and in a few days the stems collapsed and numerous small black sclerotial bodies appeared in the cortical tissue. Microscopical examinations of these seedlings were made from the time infection showed up until the plants were dead and dry, but no pycnidia were found. Likewise, naturally infected plants from the field have been examined microscopically in all stages of the disease, but no pycnidia have been found. On inoculated sweet potatoes held in sterile moist chambers, typical charcoal rot, as described by Taubenhaus (15), was produced.

Apparently, this fungus is identical with *Sclerotium bataticola*, or *Rhizoctonia bataticola*, to which Ashby (1) applied the binomial *Macrophomina phaseoli* (Maubl.) Ashby. However, since the writer has found no pycnidia associated with this seedling disease, it seems advisable for the present to adhere to Briton-Jones' (2) and Haigh's (4) classification of the fungus as *Rhizoctonia bataticola* (Taub.) Butler. The size of the sclerotial bodies, which show an average mean diameter of 75.4 μ, indicates that the fungus apparently belongs to the small-sclerotia or C group of Haigh (4),

which includes his isolations from beans and in which he found pycnidia produced, and may very likely be identical with or closely related to the fungus causing ashy stem blight of beans[5,6] (17).

CULTURES PATHOGENIC IN SOIL AT HIGH TEMPERATURES

Infection of jute, cotton, groundnut, and cowpea seedlings grown in soil inoculated with cultures of *Rhizoctonia bataticola* (then termed *R. solani*) was obtained by Shaw (7), and infection of bean seedlings under similar conditions has been obtained by Small (10), Briton-Jones (2), and Reichert and Hellinger (6).

During the winters of 1929–30 and 1930–31 attempts were made to produce the disease on bean seedlings under partially controlled greenhouse conditions. In general, the method employed was to grow the fungus on sterilized oats in fruit jars and, after it had thoroughly permeated the oats, the contents were uniformly mixed with steam-sterilized soil, which was then placed in 6-inch pots. Beans were planted in the pots of soil. Although several hundred seedlings were grown under the above conditions during the two seasons, only a few slight cases of the disease developed. During the period of the above trials, the temperature ranged from 60° to 70° F., except for a few hours during the middle of the day, when the sunlight produced a somewhat higher temperature in the glass house.

Following the occurrence of the seedling stem blight again during and immediately after a period of abnormally high temperature in 1931, it appeared quite probable that high temperature played an important part in the occurrence of the disease. On July 22, 1931, cultures of *Rhizoctonia bataticola*, prepared as previously described, were uniformly mixed into steam-sterilized soil and 24 8-inch pots were filled approximately half-full of the inoculated soil. The 24 pots were divided into 2 groups and 12 to 15 beans of the Red Kidney variety were placed on the surface of the soil of each pot in one group of pots and a similar number of the Red Mexican variety in the other group. The seed was then covered with inoculated soil to the depth of 3 to 4 inches and the pots were placed in large galvanized pans on the south side of the greenhouse. A similar series of 12 pots was prepared from noninoculated soil for controls and placed in other pans near-by. Enough water was added to the pans at intervals to maintain the water level high enough for normal growth of the plants. When the pots were placed in the pans, thermometers were placed at 1-inch and 3-inch levels in the soil. Temperature readings were taken daily from these thermometers at 8 to 9 a. m., 12 to 1 p. m., and 4 to 5 p. m. There was no apparent difference in susceptibility between the Red Mexican and Red

[5] See footnote 4.

[6] See footnote 3.

Kidney varieties and they are not considered separately in the discussion to follow.

The number of plants in each series was recorded on August 10 and it was found that only 6 plants had emerged from the soil in the 24 pots of inoculated soil. By digging into the soil, it was found that the majority of the seeds had germinated, but had been killed before reaching the surface. The 6 plants emerging from the inoculated soil died shortly after coming through the ground and only one lived long enough to develop primary leaves. All showed unmistakable symptoms of stem blight and isolations yielded cultures of *R. bataticola*. There were 86 healthy plants in the 12 pots of noninoculated soil.

On August 10, seed of the same varieties of beans was again planted in all of the pots of inoculated soil and 6 pots of the control series. On August 20, 171 young plants were up in the 24 pots of inoculated soil and 37 showed stem-blight symptoms, while all of the 50 plants in the 6 pots of the control series were healthy. By August 22, 88 additional plants in the inoculated series had developed the disease, and by August 27 only 5 plants remained alive, 4 of which died within the next 4 days. A total of 201 out of 202 plants in the 24 pots of inoculated soil were killed by the disease (Table 1). All of the plants in the control series were vigorous and healthy on September 14, when the experiment was discontinued.

During the period July 22 to August 10 when the first test was being made, the mean air temperature, as recorded by the official weather station at Davis, ranged between 70° and 85° F. with an average mean of approximately 76° F. The average daytime soil temperature, determined by averaging the 3 readings of the triplicate thermometers at each depth, ranged between 95° and 113° F. at 1-inch depth and 81° to 95° F. at the 3-inch depth. (Fig. 5.)

For the period August 10–27, when the second series of inoculation tests were in operation, the mean air temperature ranged between 69° and 83° F. with an average mean of approximately 76° F. The average daytime soil temperature at the 1-inch depth ranged between 98° and 107° F., while at the 3-inch depth the variation was 82° to 89° F. (Fig. 5.) Thus, it will be seen that relatively high temperatures prevailed during these two series of inoculations in both of which approximately 100 per cent infection was secured.

In order to test further the effect of temperature on the occurrence of the disease, the same series of pots, which had not been disturbed, were again planted on September 14 with the same varieties of beans. Ten days after planting, 468 plants were up in the 24 pots of diseased soil and only 2 showed lesions. The experiment was continued until October 21 and a

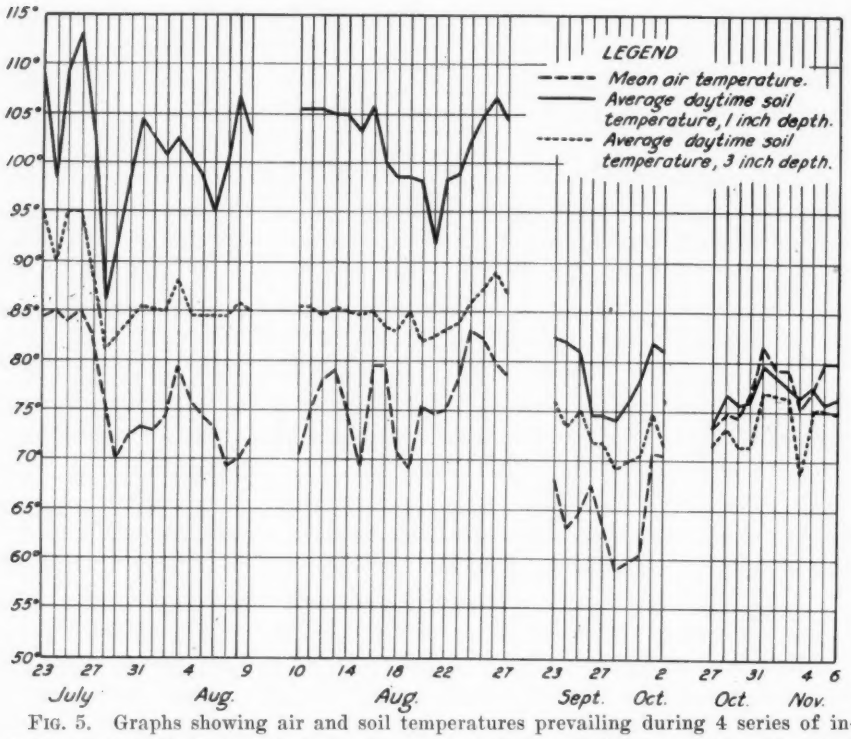

FIG. 5. Graphs showing air and soil temperatures prevailing during 4 series of inoculation tests with *R. bataticola* on bean plants, Davis, California, 1931. Abundant infection occurred during the first 2 series when high temperatures prevailed. Mean air temperatures taken from records of local office of U. S. Weather Bureau. Soil temperatures represent average of these readings at about 8 a. m., 12 m., and 4:30 p. m., each day.

total of 37 of the 468 plants showed the disease, while 209 plants came up and grew normally in the noninoculated soil (Table 1). The progress of the disease during this experiment was very slow, only a few additional plants showing the disease from day to day. The lesions enlarged very slowly and very few of the plants were killed before the experiment was discontinued. During this experiment the mean air temperature was between 58° and 71° F., with an average mean of 65°; the soil temperature at a depth of 1 inch averaged between 74° and 82° F.; while the soil temperature at a depth of 3 inches averaged between 69° and 76°. (Fig. 5.) Thus, it will be seen that the temperatures were considerably lower than during the 2 earlier experiments.

As a further test, 12 of the pots of diseased soil and 12 pots of noninoculated soil were removed on October 21 to a greenhouse, placed in large pans on a bench, and seed was planted in the same way as in previous trials.

A total of 232 plants were produced in the pots of inoculated soil and only one plant showed the disease (Table 1). A very small canker developed at a cotyledon scar on this plant. There were 219 plants in the pots of noninoculated soil and all were healthy. The mean air temperature in the greenhouse during the time of the experiment ranged between 52° and 65° F., with an average mean of 61.2°; the soil temperature at a depth of 1 inch averaged between 72° and 80° F., and at a depth of 3 inches between 69° and 77° (Fig. 5).

TABLE 1.—*Results of inoculation tests at different temperatures in 1931. Seed beans were planted in pots of sterilized soil inoculated with a pure culture of R. bataticola*

Duration of test	Temperatures	Variety	Inoculum	Number of plants	Percentage diseased
	° F.				
Aug. 11 to Aug. 27	Air[a] 76.5	Red Mexican	*R. bataticola*	99	100
			none	25	0
	Soil[b] 98–107	Red Kidney	*R. bataticola*	103	99
			none	25	0
Sept. 23 to Oct. 2	Air 65	Red Mexican	*R. bataticola*	300	8.6
			none	125	0
	Soil 74–82	Red Kidney	*R. bataticola*	168	10.1
			none	84	0
Oct. 27 to Nov. 6	Air 61.2	Red Mexican	*R. bataticola*	141	0
			none	120	0
	Soil 72–80	Red Kidney	*R. bataticola*	91	1.1
			none	99	0

[a] Average mean daily temperature.

[b] Average daytime soil temperature at depth of one inch.

The effect of temperature on the growth of the fungus in pure culture was obtained by growing the fungus in Petri dishes of neutral potato-dextrose agar at the following temperatures: 39, 46, 54, 63, 68, 77, 86, and 104 degrees F. No growth took place at 39° and in one culture a trace of growth occurred at 46°, but no sclerotial bodies were formed. At 54°, growth and sclerotia formation were very slow and meager, while at 63°, fair growth took place and many sclerotia were formed in older cultures. There was good growth at 68° and 77°, the rate of growth and sclerotia formation being somewhat more rapid at 77°. Maximum growth and rapidity of sclerotia formation in these tests took place at 86°, while at 104° only a trace of growth occurred.

The results of these inoculation tests show that *Rhizoctonia bataticola* may cause a destructive stem blight of seedling beans and thus corroborate the theory suggested by the field observations, *i.e.*, that high temperatures are necessary for infection. Apparently the disease is favored by a daytime temperature of 95° F. or above in the surface inch of soil.

SUMMARY

Rhizoctonia bataticola causes a destructive stem blight of Red Mexican, Red Kidney, and Pink bean seedlings, which occurs in epiphytotic form in the central valleys of California. The disease may kill a considerable percentage of the seedlings and seriously decrease the stand.

Seedling stem blight is characterized by black, somewhat sunken lesions on the upper part of the hypocotyl, which may involve the epicotyl and plumule and promtly kill the plant. Plants not killed in the seedling stage usually are broken over later at the point where the canker has weakened the stem.

Infection takes place on the hypocotyl at the base of a cotyledonary petiole before or soon after the seedling emerges from the soil.

The fungus, *Rhizoctonia bataticola*, has been consistently isolated from diseased seedlings. No pycnidia have been found.

Epiphytotics occurred in 1929, 1931, and 1932 in fields where the seedlings came up during and immediately following periods of about 10 days or more in which the mean air temperature was 80° F. or more, with maximum daily temperatures near or above 100° F. There were no such periods in 1930 and no epiphytotics were noted.

Bean seedlings, grown in pots of sterilized inoculated soil, became infected in a manner typical of the disease as it occurred in the field during 2 series of tests in June and July, 1931, when the daytime soil temperatures averaged 95°–113° and 98°–107° at 1 inch and 81°–95° and 82°–89° F. at 3 inches, but not during 2 later series in September and October, when the daytime soil temperatures at a depth of 1 inch averaged 74°–82° and 72°–80° and at 3 inches, 69°–76° and 69°–77° F.

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ISOLATION OF YELLOW-MOSAIC VIRUSES FROM PLANTS INFECTED WITH TOBACCO MOSAIC

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INTRODUCTION

The mosaic disease of tobacco, as is well known, is characterized by a blotching of the leaves with light and dark green areas. These areas produce the mottling or mosaic pattern, from which the disease derives its name. In addition to this mottling, small bright yellow spots occasionally occur on the leaves. They are few in number and may be easily overlooked. They are found, however, on most tobacco plants at some time in the course of systemic infection.

McKinney (4, 5, 6) has pointed out that the bright yellow areas contain a virus that differs from that of ordinary tobacco mosaic. He was able to isolate a yellow mosaic by cutting out the small yellow spots and inoculating from them. McKinney stated that the association of ordinary tobacco-mosaic virus and the yellow-mosaic virus might not constitute a contamination in the usual sense. He suggested that the yellow-mosaic virus might have arisen as the result of a mutation, although a satisfactory interpretation could not be given at that time because so few data were available. Dufrénoy (1) found that juice from bright yellow areas produced local yellow lesions when inoculated into mature leaves of tobacco.

Experiments were undertaken to determine whether the virus contained in the bright yellow spots is present in the original inoculum, occurs as the result of contamination, or arises in tissues invaded by the disease. The purpose of this paper is to describe the experiments and to report the results obtained.

MATERIALS AND METHODS

The tobacco-mosaic virus used in these experiments was of the ordinary field type and produced typical tobacco-mosaic symptoms.

The plants used in studies on bright yellow spots were *Nicotiana tabacum* L. variety Turkish, *N. sylvestris* Spegaz. & Comes, and *Lycopersicon esculentum* Mill. *N. glutinosa* L. was used in attempts to obtain samples of tobacco-mosaic virus free from all other viruses. *N. langsdorffii* Schrank was used in attempts to isolate pure samples of yellow-mosaic viruses.

All plants were grown in soil in 4-inch or 6-inch porous clay pots or in shallow wooden flats. Special precautions were taken to prevent accidental

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infection of inoculated plants with other viruses. Touching the plants during watering and other greenhouse operations was carefully avoided. The greenhouses were fumigated frequently to control insects.

The following inoculation methods were used:

1. *Pot-label method.* Two small sterile wooden pot labels were used to break off and macerate a piece of diseased leaf. Transfers of virus to other plants were made by rubbing one or two small leaves with these pot labels. The advantage of this method is that it permits the easy transfer of several different viruses without contaminating the hands.

2. *Single pin-puncture method.* A sterilized #00 insect pin was inserted into that portion of a diseased leaf from which a transfer was to be made, and then into a small leaf (0.5 to 1.5 cm. long) of a healthy young tobacco plant. This method was used in transferring virus from bright yellow spots.

3. *Glass-spatula method.* A disk of diseased leaf tissue was cut out by means of a sterile cork borer and macerated on a glass slide with a glass spatula similar to that described by Samuel (7). Inoculations were made by rubbing leaves with the glass spatula. This method was used in making transfers from local necrotic lesions occurring on leaves of *Nicotiana glutinosa* and *N. langsdorffii.*

EXPERIMENTAL PROCEDURE

In preliminary experiments it was found that bright yellow spots from which a yellow-mosaic virus could be isolated occurred on leaves of *Nicotiana tabacum* variety Turkish, *N. sylvestris,* and *Lycopersicon esculentum,* whenever such plants were inoculated with tobacco-mosaic virus. The yellow-mosaic virus obtained from these yellow spots produced symptoms that differed from those of tobacco mosaic. Plants inoculated with mixtures of the virus of tobacco mosaic and that of the yellow mosaic developed symptoms that were different from those produced by either virus alone. When tobacco plants were inoculated with mixtures containing various proportions (1:1 to 1:100) of the 2 viruses, they were easily distinguished from those infected with only one virus.

It was found that the yellow-mosaic virus, rubbed over leaves of *Nicotiana glutinosa,* produced local necrotic lesions that appeared identical with those produced on this plant by tobacco-mosaic virus. When solutions containing mixtures of these 2 viruses were rubbed over leaves of *N. glutinosa,* there appeared necrotic lesions all of which looked alike. It was found, however, that when single necrotic lesions from such leaves were used to inoculate young tobacco plants, a high percentage of the plants produced symptoms characteristic of infection with one virus only. These results suggest that single necrotic lesions offer a means of separating the virus of tobacco mosaic from that of the yellow mosaic. The method was

used in attempts to obtain samples of tobacco-mosaic virus that would be free from the virus of yellow mosaic. These attempts were carried out in the following manner. Dilute solutions of the expressed juice of tobacco plants infected with tobacco mosaic were rubbed over leaves of *Nicotiana glutinosa* plants. A number of necrotic lesions were produced. Virus from isolated single lesions was transferred through a series of *N. glutinosa* plants, each being inoculated with virus from a single lesion of the preceding plant. Necrotic lesions on these plants were frequently tested for the presence of yellow-mosaic virus by inoculating young tobacco plants from single lesions. These plants always produced typical tobacco-mosaic symptoms. No bright yellow primary lesions, such as would have indicated that the virus of yellow mosaic was present, ever appeared. It is believed, therefore, that the virus of tobacco mosaic obtained from necrotic lesions was free from that of yellow mosaic.

With tobacco-mosaic virus obtained by this method, an experiment was next undertaken to determine whether yellow spots would occur on leaves of plants systemically infected and protected against contamination in the greenhouse.

Plants used in the experiment were tobacco, *Nicotiana sylvestris,* and tomato. Twenty healthy young plants of each species, which were later to be inoculated, were placed in the central portion of a greenhouse bench. Twenty similar plants, which were later to serve as controls, were placed so as to completely surround the test plants. This arrangement left the controls in the location where they would be most exposed to accidental infection, if any should occur.

All plants were kept under close observation for at least a week before being inoculated. Neither symptoms of tobacco mosaic nor chlorotic spots of any kind appeared on the plants during this preliminary observation period.

Inoculations on the twenty plants in the center of the group were made with virus from single necrotic lesions on *Nicotiana glutinosa* leaves. The tobacco and tomato plants were inoculated from lesions obtained on the 10th serial transfer, whereas plants of *N. sylvestris* were inoculated from lesions obtained on the 15th serial transfer. The glass-spatula method was used in making the inoculations. Of the 20 plants of each species inoculated, infection was obtained in 16 tobacco, 20 *N. sylvestris,* and 10 tomato plants. All of the 60 control plants remained healthy during the course of the experiments.

Within 10 days to 2 weeks after inoculation, some of the mottled leaves of the infected plants showed small bright yellow spots, similar to those observed in previous experiments. Other yellow spots continued to appear as long as the plants were held. Typical bright yellow spots are shown in

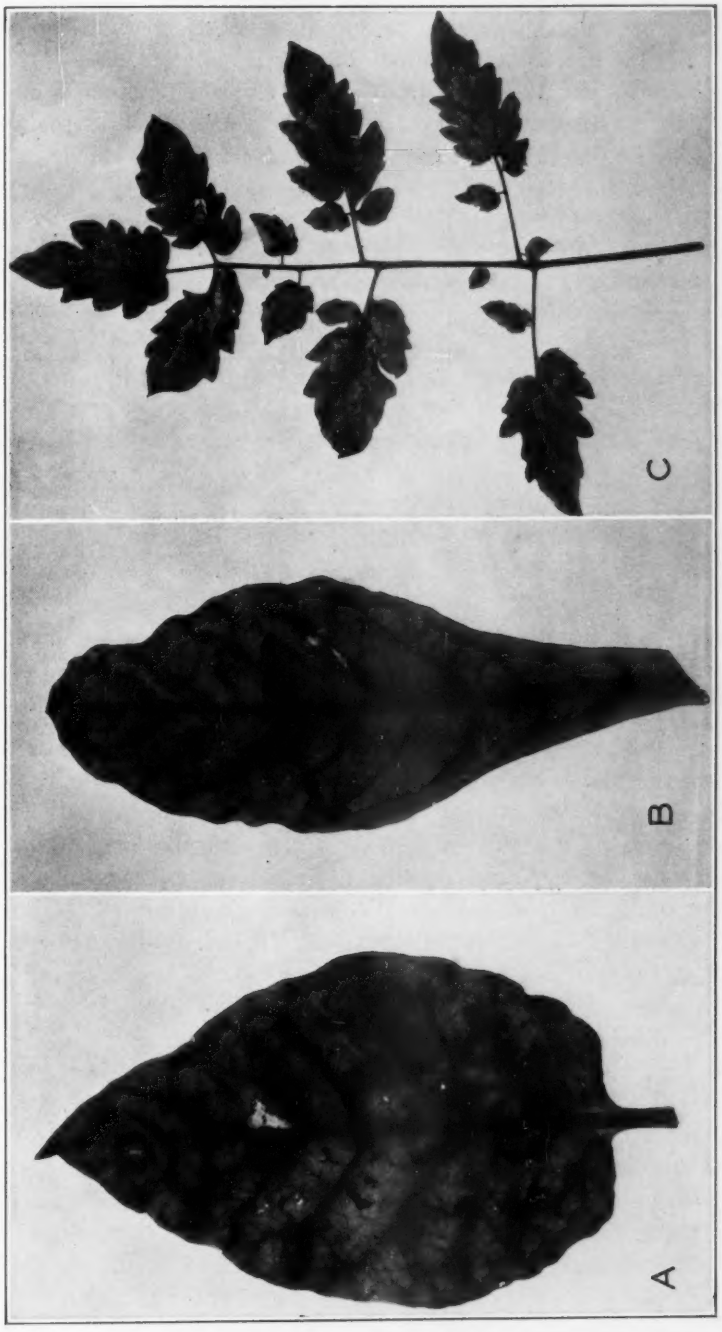

FIG. 1. Leaves of plants infected with tobacco mosaic, showing bright yellow spots similar to those from which yellow-mosaic viruses were obtained. A. Tobacco. B. *Nicotiana sylvestris*. C. Tomato.

figure 1. Of the 46 plants infected, all except 2 (1 of *Nicotiana sylvestris* and 1 of tomato) finally produced yellow spots. A total of 157 spots appeared during the period of 1 month following inoculation. Table 1 gives a summary of the results obtained. Since no chlorotic spots appeared on the 60 control plants in this experiment, it is concluded that the yellow spots on infected plants were not the result of contamination or of accidental infection.

TABLE 1.—*Summarized results of attempts to isolate virus from yellow spots in the leaves of Nicotiana spp. and tomato*

	N. tabacum	*N. sylvestris*	*L. esculentum*
Plants inoculated with tobacco-mosaic virus from necrotic lesions	20	20	20
Number of plants infected	16	20	10
Plants with yellow spots on leaves	16	19	9
Total number of yellow spots on infected plants	68	54	35
Isolations attempted	36	36	25
Yellow-mosaic viruses obtained	12	8	6

During the course of the studies, attempts were made to isolate virus from a number of the yellow spots. Leaves bearing yellow spots were removed from the plants by means of sterile scalpels. The single pin-puncture method was used in attempts to transfer virus from the yellow spots to young tobacco plants. Three to 5 days after inoculation, some of the small plants usually showed symptoms of infection. Six to 7 days after inoculation, the virus from each small diseased plant was transferred by the pot-label method to several healthy young Turkish tobacco plants. The symptoms on these plants were carefully observed. Plants showing infection with tobacco mosaic or with a mixture of tobacco mosaic and yellow mosaic were discarded. Those showing only symptoms of yellow mosaic were held separately. Twenty-six isolations of yellow-mosaic virus were made from yellow spots.

When the symptoms produced by inoculating plants from the yellow spots were studied, it became apparent that a number of different yellow-mosaic viruses had been obtained. It was observed that, while all plants inoculated with virus obtained from different yellow spots produced more or less similar primary yellow lesions, some produced distinctly different systemic symptoms. These observations suggested that the different yellow spots contained different viruses. Since these viruses were not the result of

contamination or accidental infection and, since their presence in the samples of tobacco-mosaic virus used for inoculation could not be demonstrated, even though repeated tests were made, it appears that they must have arisen in the infected plants.

For the purpose of comparing the viruses obtained from different yellow spots, attempts were made to inoculate various species of plants. Among the plants tested were the following: *Nicotiana glutinosa* L.; *N. glauca* R. Grah.; *N. rustica* L.; *N. sylvestris* Spegaz. & Comes; *N. paniculata* L.; *N. langsdorffi* Schrank; *N. acuminata* Hook.; *Lycopersicon esculentum* Mill.; *Physalis angulata* L.; *Solanum melongena* L. var. Hangchow Long.

Observations on the various kinds of plants inoculated with yellow-mosaic viruses showed that a number of the virus samples obtained from different yellow spots produced different symptoms on these plants as well as on tobacco.

On the basis of production of systemic symptoms in tobacco, the viruses appear to fall into 3 groups. Those in the first group produced general systemic infection with symptoms resembling tobacco mosaic but characterized by more intense yellowing. The viruses of this group all produced the clearing-of-veins symptom. This symptom was followed by the emergence of several leaves showing mottling and distortion. Such leaves were followed by 3 or 4 healthy-appearing, intensely green leaves. In many instances, these leaves showed no symptoms of disease until they were almost fully expanded, when blotch-like or ring-like yellow areas appeared. These yellow areas usually enlarged slowly until large portions of the leaves were involved. Later stages of the disease were characterized by the production of leaves that were more or less yellowed. The intensity of yellowing of the diseased leaf tissue varied with the different viruses of the group. Some viruses produced bright yellow chlorotic areas, others produced greenish yellow areas. The viruses producing the more intense yellow symptoms often affected the leaf tissue so severely that complete collapse and necrosis occurred. This was especially true of leaves involved in the clearing-of-veins symptom. Except for greater intensity of foliage symptoms the diseases in this group corresponded very closely with tobacco mosaic.

A second group of viruses produced systemic infections, which ordinarily advanced slowly and usually produced no typical clearing-of-veins symptoms. The first symptoms of systemic infection by viruses of this group consisted in the appearance of numerous small yellow spots on 1 or 2 of the young leaves. In some instances, these small spots tended to join together, forming a rough clearing-of-veins pattern; in other cases the yellow areas enlarged slowly until most of the leaf was yellowed. Symptoms appeared on leaves higher up on the stem in the form of blotch-like or

ring-like areas. Such spots, whether on the veins or elsewhere, enlarged slowly until most or all of the leaf was involved. The later stages of the disease were characterized by a slow enlargement of the existing yellow areas and the appearance of similar yellow areas on some of the younger leaves.

A third group contained a number of viruses that produced few or no symptoms other than the primary yellow lesions on the inoculated leaf. Some of them produced isolated yellow spots on 1 or 2 leaves above the inoculated leaf. Others produced chlorotic oak-leaf patterns, which appeared first on the leaves at the base of the stem and, later, on some of the leaves above. Several different virus samples were observed to produce no symptoms other than those on the inoculated leaf during a period of about a month following inoculation. The yellow lesions at the point of inoculation usually enlarged slowly until the entire leaf became yellow and finally collapsed. Several samples of the viruses produced yellow primary lesions that soon became necrotic.

The viruses of the 3 groups just described differ in the ease with which they are transmitted to healthy plants. Those of the first group are transferred readily, whereas those of the other 2 groups are transferred with considerable difficulty. Some of them are so difficult to transmit mechanically that even severe rubbing of the leaves with undiluted virus extracts does not give a high percentage of infection.

The yellow-mosaic viruses that have been isolated have not yet been sufficiently studied to justify a detailed description of symptoms. Brief descriptions of the most characteristic symptoms of 3 of them in tobacco and a few other plants are given below.

1. *Yellow mosaic, isolation No. 102.* This virus, which belongs in the first group, produces large yellow primary lesions on inoculated leaves of tobacco plants. These are followed by a sharply defined yellow vein-clearing and later by systemically infected leaves that are almost entirely yellow. A leaf of this type is shown in figure 2, A. Systemic symptoms of yellow mosaic are also caused by this virus on tomato, *Nicotiana sylvestris*, *N. glauca*, *N. paniculata*, and *S. melongena* var. Hangchow Long. Local necrotic lesions are produced on *N. glutinosa*, *N. acuminata*, and *N. langsdorffii*. Systemic necrosis is produced in *N. rustica* and *Physalis angulata*. The symptoms of the disease caused by this virus appear to be similar to those of Tobacco Mosaic No. 6 of Johnson (3), White Mosaic (2), and Aucuba Mosaic (8).

2. *Yellow mosaic, isolation No. 3.* This virus belongs in the third group. It produces large yellow primary lesions of indefinite outline when inoculated into tobacco leaves. These lesions continue to enlarge slowly, extending along the principal veins, as shown in figure 2, B. Usually, no symp-

FIG. 2. Leaves of tobacco plants showing symptoms of yellow-mosaic viruses. A. Yellow mosaic, isolation No. 102, mottled leaf. B. Yellow spot, isolation No. 3, primary lesions. C. Necrotic spot, isolation No. 107, primary lesion.

toms other than the slowly enlarging spots appear on the plants. The spots finally extend over most of the affected leaf until, after several weeks, the entire leaf collapses. Large yellow spots, few in number, appear occasionally on 1 or 2 leaves above the inoculated leaf, but no vein-clearing or other symptoms of general systemic infection are to be observed. In one experiment, in which 15 Turkish tobacco plants infected with this virus were held about a month, symptoms appeared on leaves other than those inoculated in only 2 plants. This virus produces yellow blotch-like primary lesions on *Nicotiana sylvestris*, tobacco, and tomato; local necrotic lesions on *N. glutinosa* and *N. acuminata*. The virus was transferred to healthy plants with difficulty. So far as is known, the symptoms of the disease caused by this virus on tobacco have not been previously described.

3. *Yellow mosaic, isolation No. 107*. Primary lesions appear on tobacco leaves 4 to 5 days after inoculation with this virus, which also belongs in the third group. These lesions are of indefinite outline, grayish yellow, and soon break down to form blotch-like grayish brown necrotic areas (Fig. 2, C). Several weeks after inoculation, small brown necrotic spots and faint, slightly chlorotic, oak-leaf patterns appear on older leaves near the base of the inoculated plants. This virus produces local necrotic lesions on *Nicotiana sylvestris*, *N. glutinosa* and *N. acuminata*. It is difficult to transfer it to healthy plants. So far as is known, the disease resulting from infection by this virus has not been previously described. The difference between it and the viruses producing yellow spots is regularly shown when inoculations are made into similar plants in parallel experiments. It is possible that the virus would not produce necrosis on tobacco under all environmental conditions. In experiments conducted during the spring and summer months under varying light and temperature conditions, it invariably produced the symptoms described.

From these brief descriptions it is seen that the 3 viruses produce distinctive symptoms. Some of the other yellow-mosaic viruses produce less striking differences.

To test the possibility that the small differences might be due to mixtures, many of the viruses were reisolated from single necrotic lesions on leaves of *Nicotiana langsdorffii* in the following way. Each yellow mosaic was transferred to a separate set of *N. langsdorffii* plants. Six or 7 days after inoculation, 10 single necrotic lesions were cut out of the leaves of each set and used to inoculate 10 tobacco plants, each lesion being used on a different plant. In every instance, the 10 inoculated tobacco plants of a set gave identical symptoms. It was, therefore, concluded that the viruses thus tested were free from other viruses and that the differences observed resulted from differences inherent in each of them.

DISCUSSION

Evidence obtained during the course of this investigation indicates that yellow-mosaic viruses arise in bright yellow spots that regularly appear on the leaves of tobacco and tomato plants affected with tobacco mosaic. Some of these viruses cause diseases that differ markedly from each other and remain distinct in serial transfers. Others produce symptoms that differ so slightly that they are almost indistinguishable on the plants to which they have been transmitted. Still others cause diseases that seem identical.

It is believed that the possibility that these viruses may be carried along with the virus of tobacco mosaic in transfers from plant to plant or that they may be accidentally transmitted from some other plant to the tissues in which they appear, has been eliminated. Some of them are difficult to transmit to tobacco. Considering this characteristic, it seems improbable that they would be carried along in ordinary tobacco-mosaic inoculation. Since it is very difficult to recover them from the necrotic lesions they produce on *Nicotiana glutinosa,* it is even more improbable that they would be retained through a series of single necrotic-lesion transfers of tobacco mosaic such as was made in efforts to obtain pure samples of this virus. No bright yellow spots have ever been observed on uninoculated check plants. They occur only on plants that have tobacco mosaic and are far too numerous to permit their being considered as due to accidental contaminations. It is, therefore, believed that the yellow-mosaic viruses arise from the tobacco-mosaic virus. Although these viruses and the diseases they produce have not yet been sufficiently studied to justify conclusions regarding the extent to which they differ from each other or from the tobacco-mosaic virus, they are considered to be strains of the latter. All of them are readily distinguished from the latter by the symptoms produced in tobacco. Some also differ in showing a lower infectivity and a slower rate of spread from primary lesions. Insofar as has been been determined, they have the same host range as tobacco mosaic. In several different plants they produce necrotic primary lesions that are indistinguishable from those of the latter disease. Nothing is known as to the manner in which they arise, or the conditions favoring their production.

Some of the yellow mosaics of Solanaceous plants prevalent in commercial fields and greenhouses may have originated in yellow spots on leaves of plants having tobacco mosaic. It is possible that sucking insects that accidentally feed on the bright yellow spots on such leaves and later on healthy tobacco or tomato plants may thereby isolate yellow-mosaic viruses and start epidemics of such diseases as Tobacco Mosaic No. 6 of J. Johnson (3), the White Mosaic of E. M. Johnson (2), or Aucuba Mosaic (8). Some of the yellow mosaics isolated differ from any that have previously been described.

SUMMARY

Small, bright yellow spots, containing the viruses of yellow mosaics, occasionally appear on leaves of *Nicotiana tabacum* variety Turkish, *N. sylvestris,* and *Lycopersicon esculentum* infected with tobacco mosaic.

Similar yellow spots appear when plants of these species, protected against accidental infection, are inoculated with tobacco-mosaic virus shown to be free from all other viruses.

Twenty-six isolations of yellow-mosaic virus were made from yellow spots. Many of them differ from each other. The symptoms produced by 3 that showed marked differences are briefly described.

Evidence obtained in this investigation indicates that viruses of yellow mosaics arise during multiplication of tobacco-mosaic virus in infected plants.

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SOME SIGNIFICANT ESTIMATES OF LOSSES FROM PLANT DISEASES IN THE UNITED STATES

NEIL E. STEVENS

(Accepted for publication November 15, 1932)

Any one familiar with the literature of plant pathology in the United States will recall the frequency with which publications on the cause and control of a disease have included a statement of the importance of the disease, with an estimate, often expressed in dollars, of the losses which it was believed to cause. As is generally recognized, these estimates of crop losses were little more than guesses by informed and experienced observers, while the figures given in dollars were usually obtained by merely multiplying the commercial units estimated as lost by the sale price per ton or per bushel received for that portion of the crop actually sold.

WHY "DOLLAR" ESTIMATES ARE RARELY SIGNIFICANT

Obviously so simple a method of computing dollar losses can no longer pass even for purposes of propaganda. It is now clearly realized that not all losses from disease are of equal commercial significance. The economic losses from decay of fruits and vegetables in transit and on the market are much greater than the actual percentage of loss, for they involve the costs of harvesting, packing and shipping, as well as frequent claims for damage, and often what is more serious, lowering of the general price level and slowing-up of demand.

Losses from disease in the field, on the other hand, while often large and sometimes of tragic significance, usually involve only reduction in crop. This condition while serious enough to the individual grower may not appear so to the industry as a whole. Indeed, some pathologists[1] have gone so far as to suggest that "Viewed from the standpoint of the whole industry, the presence of these diseases is perhaps indirectly a benefit in its ultimate effect, since it serves as one of the natural checks on overproduction." Obviously, the difficulties in arriving at a fair understanding of the complex economic factors involved are too great to make possible even a reasonable approximation to dollar estimates of crop losses.

THE CROP LOSS ESTIMATES COMPILED BY THE PLANT DISEASE SURVEY

With the formal organization of the Plant Disease Survey in 1916 and under the stimulus of the demand for foodstuffs occasioned by war conditions an attempt was made to collect on a national basis estimates of losses

[1] Fawcett, H. S. and H. A. Lee. Citrus diseases and their control. 582 pp. McGraw-Hill Book Co., Inc., New York. 1926. (p. 1.)

due to disease in agricultural crops. The plan of operation, which has been continued to the present time, was to assemble from working pathologists throughout the United States, chiefly from the collaborators of the Plant Disease Survey, estimates in per cent of losses due to various diseases within their localities. The data obtained from all these sources were combined and preliminary estimates were sent to collaborators and to pathologists of the Bureau of Plant Industry for their final criticism and revision.

In these tabulations the losses were expressed in percentages of the total crop and in amount (in commercial units) of reduction in yield due to diseases. Reduction in yield was calculated by considering the production for the year as 100 per cent minus the percentage of loss from disease. The average estimated percentages of loss for the United States were obtained by dividing the total reduction due to all diseases by the sum of the known production plus the estimated reduction. This method was the one then followed by the Bureau of Crop Estimates. In spite of their admitted limitations, these estimates are probably the best that can be assembled for the period and will have decided historic interest as showing which diseases were considered most important by American students of plant diseases during this decade.

Finally, in 1931, a series of maps and charts was prepared by Miss Jessie I. Wood, which included the most complete reports of some of the

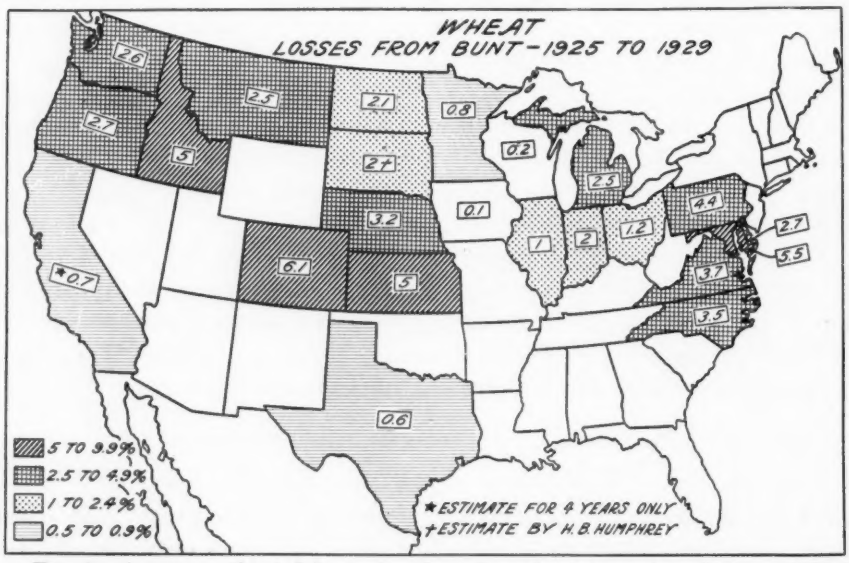

FIG. 1. Average estimated losses in wheat from bunt in various States. 1925 to 1929. States not shaded did not report regularly.

commoner and better-known diseases. These charts were distributed to pathologists for comments and suggestions. On the basis of these comments and our own study, 3 of these crop estimates have been selected that appear to be particularly significant or that can be checked by some other wholly independent method of estimating disease loss.

BUNT OF WHEAT

The estimates of losses from bunt of wheat for the 10-year period 1920–1929 are based on the reports from only 20 States and from this list some of the important wheat-producing areas are lacking. From 1925 to 1929 reports were, however, more numerous and as the reporting States are well scattered it seems probable that the figures are fairly representative. (Fig. 1.)

There is, moreover, supplemental evidence that the curve (Fig. 2) of

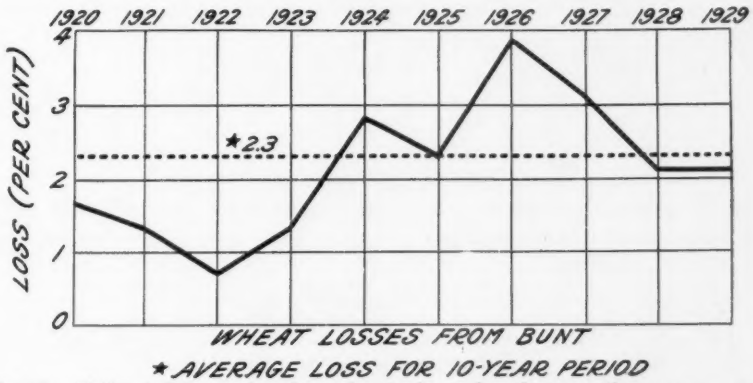

FIG. 2. Estimated losses in wheat due to bunt for the reporting area as a whole. 1920 to 1929. Average loss for the entire period is shown by the dotted line.

loss for the 10-year period is significant in its main features, that is, low losses up to and including 1923 and relatively high losses for 1924, 1926, and 1927, with somewhat lower figures for 1925, and since 1927. This evidence is found in the summary of information on "Smutty Wheat" compiled by E. G. Boerner, R. J. Haskell, and F. C. Meier. This summary consists of an analysis of the information on smutty wheat contained in the monthly reports of grain inspections for the United States as submitted by the various offices of Federal Grain Supervision of the Bureau of Agricultural Economics. The curve (Fig. 3) is made up of a simple average of the percentage of cars grading smutty at 15 important markets, from which reports are available for each of the years included. No doubt a weighted average that took into consideration the number of cars handled at each market would be more correct mathematically, but a simple average

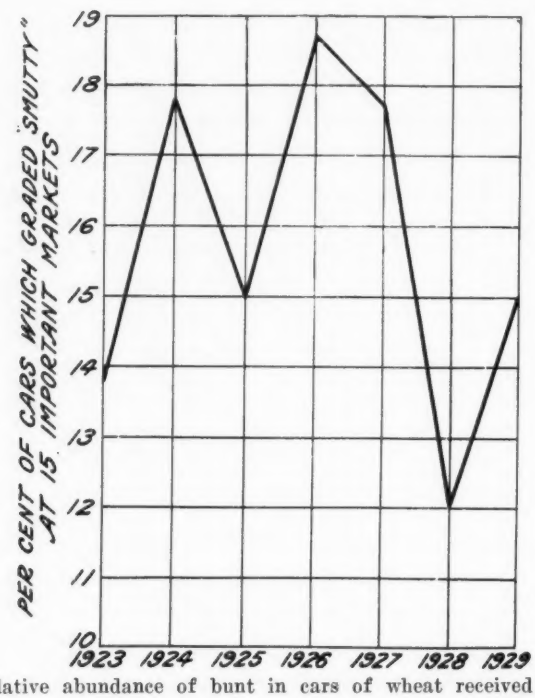

FIG. 3. Relative abundance of bunt in cars of wheat received at 15 important markets expressed as a simple average of percentage of cars which graded smutty. 1923 to 1929.

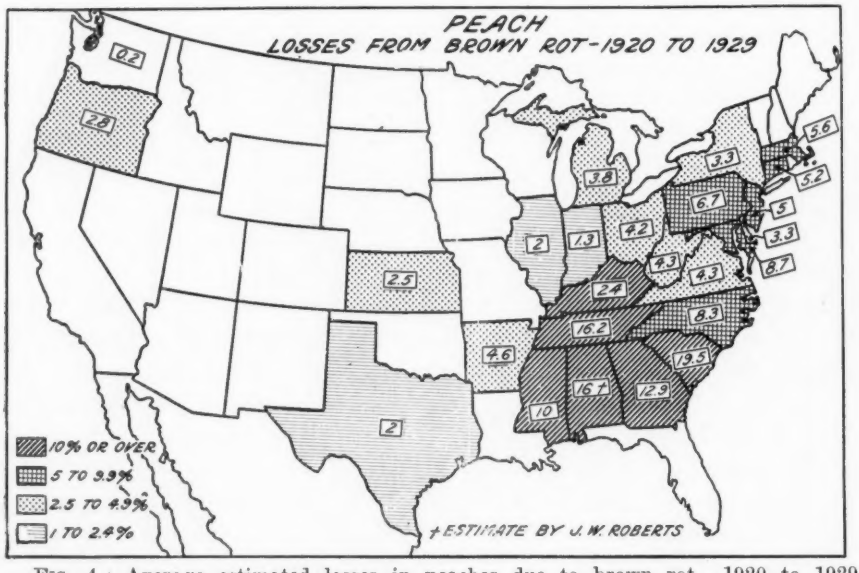

FIG. 4. Average estimated losses in peaches due to brown rot. 1920 to 1929. States not shaded did not report regularly.

shows as close a parallel as can be expected from figures derived from such widely different sources.

PEACH BROWN ROT

In the eastern part of the United States the losses from brown rot of peaches are, as is well known, much more serious in the Southern than in the Northern States. This appears on the map. (Fig. 4.)

The significance of the curves of estimated losses from peach brown rot (Fig. 5) is attested by a comparison with the curve based on the reports

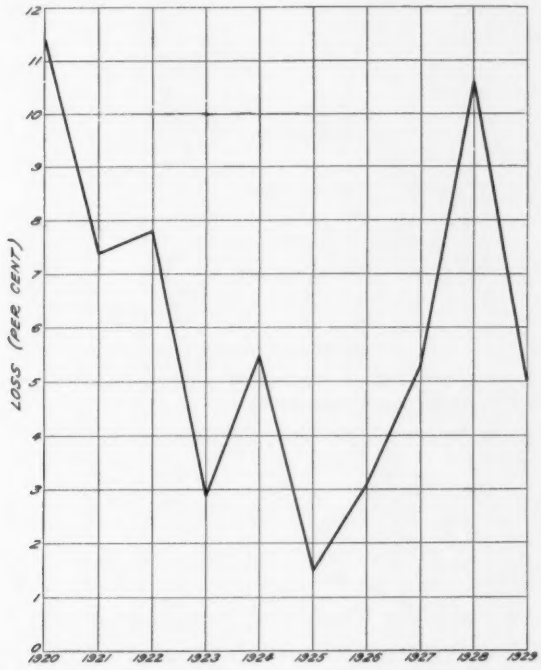

FIG. 5. Estimated loss in peaches due to brown rot in the reporting area as a whole. 1920 to 1929.

of the Food Products Inspection Service of the Bureau of Agricultural Economics. (Fig. 6.) In connection with a study of spoilage of stone fruits on the market, Brooks[2] analyzed these reports over the period of 1922–1928, inclusive, and published a careful tabulation of the results. These figures include the results of the inspection of over 7,000 cars of peaches. The actual number inspected in a single year varied from 752 in 1925 to 1,399 in 1928.

[2] Brooks, Charles. Spoilage of stone fruits on the market. U. S. Dept. Agr. Circ. 253. 1933.

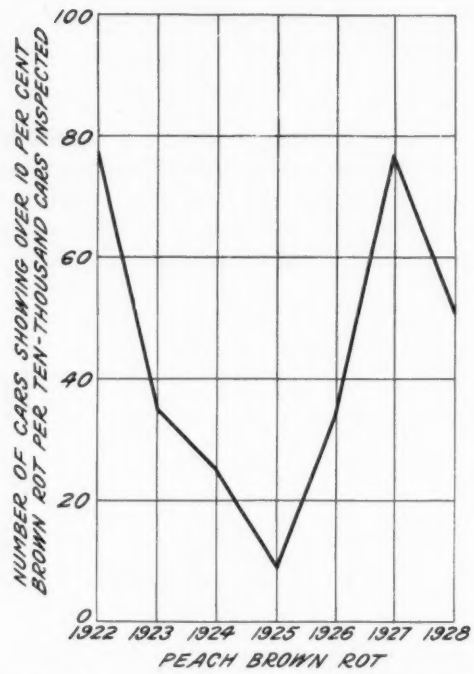

FIG. 6. Relative abundance of brown rot of peaches, 1922 to 1928, as shown by reports of the Food Products Inspection Service, expressed in the number of cars showing more than 10 per cent brown rot per 10,000 cars inspected.

The curve (Fig. 6) is based on Brooks' table and gives the percentage of cars inspected each year that showed over 10 per cent brown rot. The two curves thus deal with the same disease but are derived from wholly different sources. It must then be of significance that they show the same general trend—a sharp decline in losses from 1920 or 1922 to 1925, followed by a marked rise after that date.

CAUSES FOR THE FLUCTUATION IN BROWN ROT

Reference to the map shows the degree to which brown rot in the Southern States exceeds that farther north. Among these States, Georgia is preeminent in peach production; so great is this predominance that developments in this State overshadow all the others in the averages. J. W. Roberts' review of conditions in Georgia, which follows, appears to furnish a satisfactory explanation of the trend of the curves in figures 5 and 6.

Undoubtedly a considerable amount of the brown rot in 1920 was due to carelessness in spraying. In 1917 and 1918, in Georgia, there was almost no brown rot and no curculio. The impression became very general among growers that these pests had been practically eliminated. Spraying in 1919

and 1920 was of the most casual nature, and in 1920 both brown rot and curculio were very bad. In 1921, a serious spraying campaign was undertaken, the effects of which are plainly evident during the succeeding years, 1921 to 1926, inclusive. With the coming, however, of lower prices for peaches and the severe economic conditions of 1927 and the following years, there was a decided reduction in control activities and a corresponding increase in disease. (Unpublished memorandum.)

STORAGE ROTS OF SWEET POTATO

Losses to sweet potatoes from decay in storage have been the subject of careful study and have long been recognized as important. This was the only crop in which losses in storage were reported in sufficient detail to warrant compilation. The map (Fig. 7) is based on the reports of col-

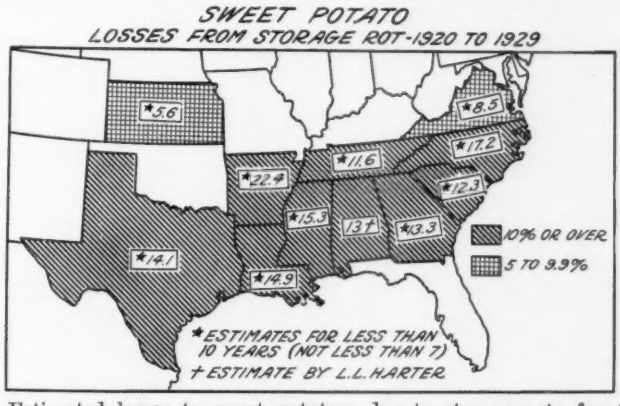

FIG. 7. Estimated losses to sweet potatoes due to storage rots for the reporting area as a whole. 1920 to 1929. States not shaded did not report regularly.

laborators, except for Alabama, which was interpolated on the advice of L. L. Harter.

The curve of storage losses (Fig. 8) apparently furnishes an example of the significance of extension work in plant pathology where effective control measures are known. Reduction of storage losses in sweet potatoes may be brought about by careful handling and drying of the potatoes after they are harvested, and the maintenance of proper temperature and humidity in the storage house. Storage losses also are materially reduced by the elimination of black rot and other diseases as a result of seed selection, seed treatment, and the application of other sanitary measures.

By 1920 the seriousness of the losses was fully recognized and the methods of control well known. An intensive campaign for their reduction, led by Dr. Harter, and no less effective for not being formally organized, was begun by Federal and State pathologists at this time. Early in 1922, F. C. Meier was made Extension Pathologist and took up the reduction

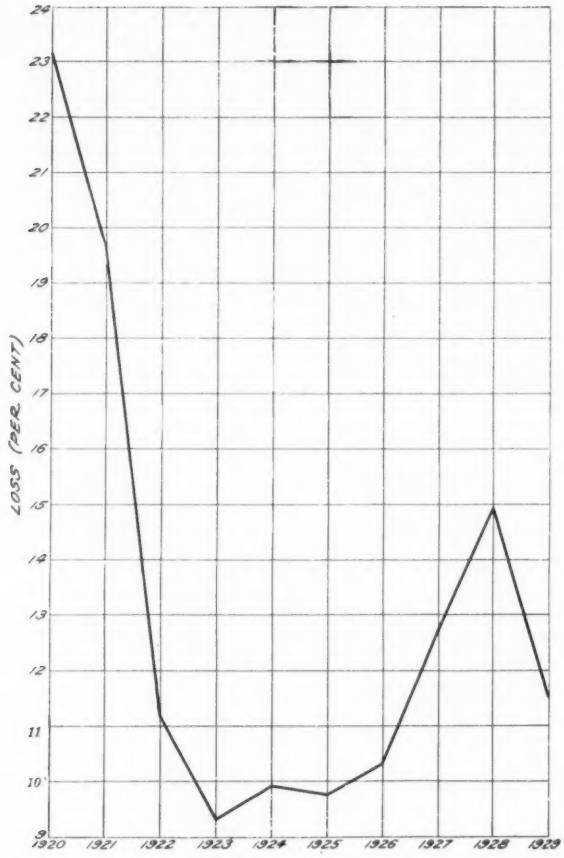

FIG. 8. Average estimated losses in sweet potatoes due to storage rots. 1920 to 1929.

of losses from rot in storage of sweet potatoes as one of his main projects. The result of these combined efforts was that in the 3-year period, 1920 to 1923, losses were reduced more than one half. The low level was maintained essentially unchanged for several years.

It should not be supposed that 1920, the first year shown on the chart, was characterized by unusually high losses from storage rot of sweet potatoes; in fact, losses estimated for that year are lower than for any of the few preceding years available. The average estimated losses for 1917, 1918, and 1919 were almost 28 per cent as against 23 per cent for 1920. In other words, the reduction shown on the chart indicates a new level as compared with that of the earlier period.

By 1926, however, Meier had diverted much of his attention to wheat-smut control, Harter had undertaken a study of bean diseases as his chief

project, and the low price of sweet potatoes had reduced the interest of the growers. A general increase in losses from storage rot followed.

ESTIMATES OF LOSSES FROM DECAY IN FOREST PRODUCTS

The emphasis here placed on estimates of losses from a few diseases that may be checked in more than one way should not be interpreted as meaning lack of interest in other types of estimates. Almost any sincere estimate by an informed observer has at least comparative value. Certain types of losses of great interest to pathologists are more easily measured than losses to growing crops. Examples of this are the losses by decay of fruits and vegetables in transit, which have been made the subject of several special studies, and even more conspicuously the losses from decay in certain forest products. Colley[3] has devoted to this subject a considerable amount of study, the results of which are largely unpublished, but certain of his estimates quoted below are typical.

TABLE 1.—*Estimated annual loss resulting from decay in forest products*

Estimated quantity of timber removed from forests of the United States		Estimated annual loss from decay	
Kind of material	Equivalent in standing timber in cubic feet	Equivalent in standing timber in cubic feet	Approximate per cent
Ties, sawed and hewed	1,320,000,000	660,000,000	50.0
Fence posts	1,800,000,000	900,000,000	50.0
Poles	55,250,000	27,625,000	50.0

Most of the other estimates in the same table are of almost equal significance but those given will serve as examples.

TOTAL YIELD AS AN INDICATION OF LOSS FROM DISEASE

Ordinarily so many factors other than disease, such as acreage and weather enter into total yields that it is not possible to relate yield directly to disease losses. There has recently been published, however, a report on cranberry production in New Jersey, which seems to be of direct significance in connection with the incidence of disease.

The smoothed curve of total cranberry yields (Fig. 9) in New Jersey prepared by Wilcox, but not published in his paper, indicates a fairly con-

[3] Colley, Reginald H. Utilization and conservation of the timber supply. Science, n. s. **61**: 107–109. 1925.

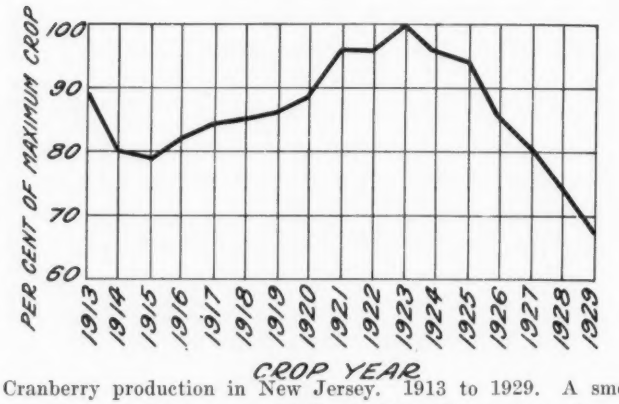

FIG. 9. Cranberry production in New Jersey. 1913 to 1929. A smoothed curve plotted in terms of the maximum crop of the period. The decline since 1923 is due almost entirely to the inroads of the false-blossom disease.

stant increase in yield from 1915 to 1923 followed by rapid and continued decline after that time. The significance of the production curve has been discussed by Wilcox,[4] and it is necessary here only to point out that the increase up to 1923 was largely due to the coming into bearing of newly planted areas, combined with the improved control of insects and fruit rots, and that the decline since 1923 has been chiefly if not solely due to the inroads of the false-blossom disease, particularly on comparatively recent plantings of Howes and older areas of native Jerseys.

The continued decline over a period of 8 years in the production of a standard fruit crop in the face of satisfactory, even increased, prices for the product furnishes perhaps the most striking possible evidence of the severity of this particular disease. To find a parallel it would be necessary to go back to the even more serious inroads of peach yellows in the Delaware Peninsula and the Blue Ridge regions in the last decade of the nineteenth century.

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[4] Wilcox, R. B. Adjustments to false blossom in New Jersey. Proc. Amer. Cranberry Grow. Assoc. 63: 7–11, 14–15. 1932.

OBSERVATIONS ON TOLYPOSPORIUM FILIFERUM, CAUSE OF "LONG SMUT" OF SORGHUM[1]

M. N. KAMAT

(Accepted for publication March 8, 1933)

The long smut of sorghum (*Tolyposporium filiferum* Busse) was first described from East Africa in 1904 by Busse (3), who collected it from various parts of Tanganyika Territory, where it apparently did little damage. Barber (1) probably had this smut in mind when he described 3 smuts as occurring in the Madras Presidency, India, as the symptoms he

FIG. 1. Gross effects produced by *Tolyposporium filiferum* on sorghum. Note the long sori.

[1] The work reported in this paper was done in the laboratory of Plant Pathology, University of Minnesota. The writer is grateful to Dr. E. C. Stakman for the facilities and help afforded him during the investigation.

described coincide with those produced by this smut. Kulkarni (5) reports long smut from Bombay, India, and states that it is confined mostly to the extreme northern sections, particularly Sindh, but sometimes occurs in Kathiawar. He states that it causes heavy losses locally in certain years, although, in general, it is not considered a destructive disease.

Thomas (7) mentions the occurrence of long smut in Mesopotamia, and, according to Briton-Jones (2), it is common in Egypt. It has not yet been reported from the United States. Figure 1 shows the gross effect produced by this disease on sorghum.

Although long smut has been studied by Busse and Kulkarni, many essential points are lacking in our knowledge of the reaction of the pathogen to its environment. The investigation reported in the present paper includes a study of certain factors that influence the germination of chlamydospores, and it also includes a study of cultural characteristics of the of the organism on nutrient media.

SPORE GERMINATION

Kulkarni (5) obtained germination in water, dung solution, tomato broth, and glucose-peptone solution. Busse (3), on the other hand, reports germination only in the last solution. While Kulkarni (5) reports germination by both promycelium and germ tube, his figures illustrate only the former type. Busse (3) reported formation of long chains of aerial conidia

TABLE 1.—*The percentage and type of germination of chlamydospore clusters of Tolyposporium filiferum on several kinds of solid and liquid media at 28° C.*

Medium	No. of spore balls	Percentage of germination		
		By promycelium	By direct tube	Total
1 per cent potato-dextrose agar	81	14	82	96
Dung agar	78	12	53	65
Carrot agar	102	33	57	90
Nonnutrient agar	72	4	79	83
One per cent lactose	255	50	12	62
One per cent maltose	279	46	24	70
One per cent dextrose	276	48	13	61
Knop's solution	110	.....	.....	tr.
Sterilized distilled water	375	86	2	88

on the surface of a culture. Since extensive observations have not been recorded, the writer made a detailed study of germination.

Material for study was obtained from Bombay, India,[2] collected from the 1931 crop in Sindh. Germination tests were made both in Syracuse dishes and in hanging drops. The latter method was exclusively used for solid media. This was done by picking chlamydospore clusters from a dry coverslip and placing them on drops of various solid media with the help of a Zeiss micromanipulator. Observations and counts were made at the end of 48 hours. All the germination studies were made at 28° C. Several media were tried and the data obtained are tabulated in table 1.

The chlamydospores usually germinated readily on the solid and liquid media, and in sterilized water. All the chlamydospores in a spore ball frequently germinated but isolated individual chlamydospores germinated very capriciously. In the numerous trials made not more than 10 per cent of isolated single chlamydospores germinated.

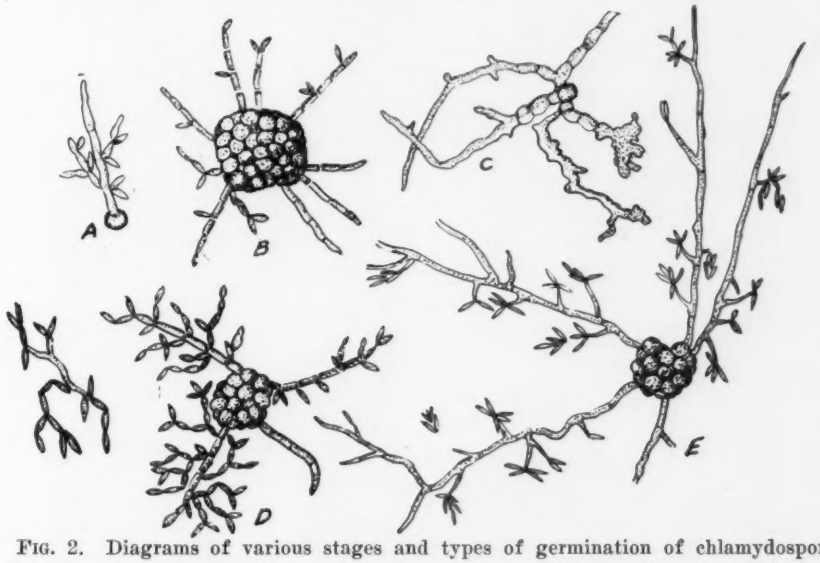

FIG. 2. Diagrams of various stages and types of germination of chlamydospores of *Tolyposporium filiferum* at 28° C. A, B, and D in sterile distilled water and C and E on 1 per cent potato-dextrose agar.

It is evident from table 1 that the percentage of germination on dung agar was much lower than on the other solid media, possibly because of its higher acidity. Germination was much more vigorous, as measured by the length of tubes, on 1 per cent potato-dextrose agar, carrot agar, and on the nonnutrient agar. As a rule, typical promycelia and sporidia were formed

[2] The writer is grateful to Dr. B. N. Uppal, Plant Pathologist, Agricultural College, Poona, India, for the supply of chlamydospores.

FIG. 3. Two types of germination in *Tolyposporium filiferum*. A. Aerial conidia borne in clusters. B. Aerial conidia borne in chains.

most often in liquid media and especially in sterilized distilled water (Fig. 2, B and D), while on solid media the chlamydospores commonly produced germ tubes, which branched laterally, forming aerial conidia, either in long chains (Fig. 3, B) or in clusters at ends of short, pointed, thorn-like branches (Figs. 2, E; 3, A). The former method of conidial formation apparently was favored by a relatively dry environment, while the latter type was induced by a moist medium.

Striking variations were noticed in the promycelial type of germination. The promycelium was generally quadricellular, but sometimes tricellular, and, in some cases, promycelia of 5 and 6 cells were observed. The sporidia were spindle-shape and from 8 to 24 μ long. They were formed both laterally and terminally, often in clusters at single points and on both sides of the promycelium; in some cases, they were formed directly from the chlamydospore, without the formation of a promycelium. The aerial conidia were much shorter and measured from 4 to 8 μ in length.

Studies were made to determine the cardinal temperatures for the germination of chlamydospores. One per cent potato-dextrose agar and sterilized distilled water were used for these tests. The results are summarized in table 2.

The data indicate that optimum temperature for germination of chlamydospores is about 28° C. However, the percentage of germination at 33° C. was almost as high as at 28° C. The minimum was between 10 and 13° C., while the maximum was a little above 39° C.

TABLE 2.—*Percentage of germination of chlamydospores of Tolyposporium filiferum in one per cent potato-dextrose agar and in sterilized distilled water at different temperatures*

Temperature in °C.	One per cent potato-dextrose agar		Sterilized distilled water	
	No. of spores	Percentage germination	No. of spores	Percentage germination
10	104	0	85	0
13	60	50	0	.....
15	70	52	92	33
20	180	59	102	69
25	82	83	148	73
28	108	91	105	82
33	120	88	98	78
36	80	31	80	19
39	75	8	65	tr.

CULTURAL CHARACTERS

Approximately 100 monosporidial lines of *Tolyposporium filiferum* were isolated and studied to some extent. The method of isolation was essentially that used by Hanna (4). The sporidia were numbered 1, 2, 3, and 4 in order from the tip of the promycelium to chlamydospore. Sometimes it was difficult definitely to locate the position, because of the clusters of sporidia produced at each point, in which case, letters a, b, c, etc., were used for designating such lines. A number also was assigned to an individual chlamydospore whenever a culture was made from it. Thus 16–1 refers to a line that originated from the tip cell of the promycelium of chlamydospore No. 16.

Several media, such as carrot agar, carrot agar + 1 per cent dextrose, nonnutrient agar, dung agar, and Brown's artificial potato agar were tried in addition to 1 per cent potato-dextrose agar. None of them promoted good growth except the last one. On carrot agar the growth was slow and the diameter of the colony small. On carrot agar to which 1 per cent dextrose was added the growth was still meager. On nonnutrient agar, the growth was very slow, flat radial, silken in texture, and white myceloid.

Figure 4 shows the comparative growth of line 16–2 in 1 per cent potato-dextrose agar and in carrot agar under standard conditions. There was a marked tendency for sectoring on carrot agar. These wedge-shape sectors were usually smooth, mycelioid, and so membranous in consistency that it

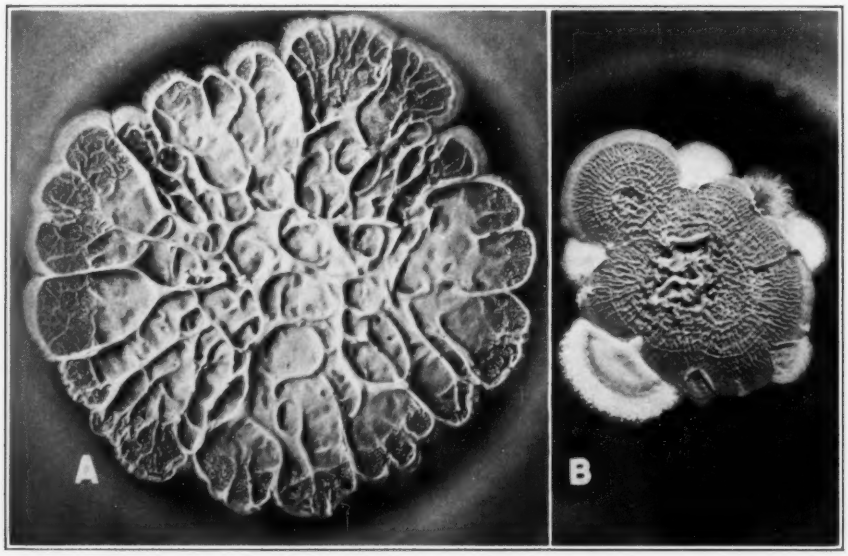

FIG. 4. Growth of monosporidial line 16–2 at end of 10 days. A. On 1 per cent potato-dextrose agar. B. On carrot agar. Note sectors.

was difficult to make transfers from such sectors except by cutting pieces with a sharp spatula.

Most of the monosporidial lines isolated showed no marked differences in their cultural characters when grown on several different kinds of media. A few, however, were distinctly different. Four of these lines and one monochlamydospore culture were selected for further study.

The cultural characteristics of 5 lines of smut on 1 per cent potato-dextrose agar are given in table 3. The apparent differences were chiefly

TABLE 3.—*Cultural characteristics of five lines of Tolyposporium filiferum grown on 1 per cent potato-dextrose agar for 9 days at 28° C.*

Line	Color[a]	Topography	Surface	Margin	Consistency	Diameter of colony in mm.
19	Pale cinnamon pink	Raised Broadly ridged	Dull	Lobate	Membranous	40.0
16–2	Buff	Raised Finely ridged	Dull	Wavy	Membranous	50.0
11–1	Pale cinnamon pink	Rugose Broadly ridged	Dull	Lobate	Leathery	50.0
20–2	Pale cinnamon pink	Raised Broadly ridged	Waxy	Lobate	Yeastoid	37.5
13–d	Ivory yellow	Raised Broadly and deeply furrowed	Waxy	Broadly lobate	Yeastoid	40.0

[a] According to color standards and nomenclature of Ridgway (6).

in color, consistency, margin, and topography of colonies. Certain lines also could be distinguished by their rate of growth at different temperatures.

The effect of temperature on the rate and type of growth of the 5 cultures on 1 per cent potato-dextrose agar was determined. Flasks of 250 cc. capacity, with 40 cc. of medium, were inoculated in duplicate and were incubated in the dark and kept at different temperatures. At higher temperatures, as a general rule, the colonies tended to be membranous in consistency, much paler in color, with less distinct and more regular margin, and the ridges were finer than those at lower temperatures. At lower temperatures, namely, between 20° C. and 25° C., the cultures were brighter in color, raised, and deeply and broadly ridged in topography; the margin was broadly lobed, and the consistency yeast-like.

Two monosporidial lines, 16–2 and 13–d, showed very marked differences in their rate of growth; line 16–2 grew much more rapidly than did line 13–d. This difference was maintained at all the temperatures in all the

trials made. The temperature range for growth of line 13–d was 13° C. to 36° C.; it was 10° C. to 39° + C. for line 16–2. The optimum temperature for line 13–d lies between 28° C. and 33° C., while that for line 16–2 is between 33° C. and 36° C.

It would appear that the optimum temperature for growth in culture is not the same as for chlamydospore germination. Lines 13–d, 19, and 20–2 showed very close agreement between the germination tests made on potato-dextrose agar, while 16–2 and 11–1 had higher optimums as well as wider growth ranges. No definite cardinal temperatures, therefore, could be determined, since there was so wide a variation between lines. In general, however, it can be said that the optimum for growth in culture lies between 28° C. and 36° C.

SUMMARY

Tolyposporium filiferum, causing long smut of sorghum, is sometimes destructive in certain parts of India, but it has not been reported from the United States.

Chlamydospores of long smut germinate readily on many different types of medium and over a wide temperature range. In water and in liquid nutrients typical promycelia are usually developed, while on solid media the chlamydospores commonly produce a germ-tube-like growth, which branches and forms aerial conidia either in long chains or clusters.

Approximately 100 monosporidial lines were isolated and studied culturally on solid nutrient media. Most of these smut lines were alike; a few, however, differed strikingly from each other in the following cultural characteristics: color, consistency, margin, surface, topography, and rate of growth of the colonies. Wedge-shape sectors were frequently observed in monosporidial lines when grown on certain nutrient media.

The influence of temperature on growth in culture varied with the different lines, but the optimum temperature for growth of all lines studied lies between 28° and 36° C.

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BOTRYTIS CINEREA AS A CAUSE OF POTATO TUBER ROT

DONALD FOLSOM

(Accepted for publication January 26, 1933)

Pethybridge (14, pp. 577–578), in 1916, while discussing the *Botrytis cinerea* blight of potato vines in Ireland, stated that this fungus does not affect the tubers. However, it is of interest that, from tubers affected with a "watery" or "glassy" rot, he sometimes isolated "pure cultures of a Botrytis which appeared to be identical with *B. cinerea*" (14, p. 591). He states that "this form of rot . . . has only been found to occur in . . . tubers which . . . remain attached to the parent plant at the time of digging," and "no infection experiments were made with the Botrytis, as previous trials with the same fungus isolated from leaves gave negative results" (14, p. 591).

The apparent immunity of the tubers is again pointed out by Cotton in 1919 (9, p. 43). Brown (8, p. 291), in 1922, related that potato-tuber tissue gives off volatile substances that retard the germination of spores of *Botrytis cinerea*. He concluded that the atmosphere of a potato storage place is very unfavorable to Botrytis germination (8, p. 298) but points out that "as regards the effect of volatile constituents of the plant on the growth of the fungus, once the latter is inside, little can be said at present" (8, p. 298).

The isolation of a Botrytis in pure culture from several soft-rotted tubers stored in Maine was reported in 1927.[1,2]

The writer in the spring of 1932, while examining potato tubers (*Solanum tuberosum* L.), of the Green Mountain variety, stored in a typical potato cellar in northeastern Maine, found tubers with apparently a new kind of rot. The exterior of the tuber showed a flabbiness and a wrinkling of the skin that were more pronounced as one approached the stem end of the tuber. (Fig. 1, A and B.) A cut through the conspicuous junction of flabby and normal solid tissue disclosed a rot that was rather soft, though not wet unless squeezed, and that was advancing in the interior and causing a temporary darkening of the flesh. (Fig. 1, B.) Where the rot had penetrated the tissues, the eyes were filled with tufts of gray mold (Figs. 1, A, B, and 2, A) with which blue mold (Penicillium) was sometimes associated. From the "frontier" of the advancing rot, pure cultures of a Botrytis were readily isolated. With these cultures and others

[1] U. S. Dept. Agr. Bur. Plant Ind., Pl. Dis. Rprtr. **11**: 54. (Mimeographed.) 1927.

[2] Diseases of vegetable and field crops (other than cereals) in the United States in 1927. U. S. Dept. Agr. Bur. Plant Ind., Pl. Dis. Rprtr. Sup. **61**: 237. (Mimeographed.) 1928.

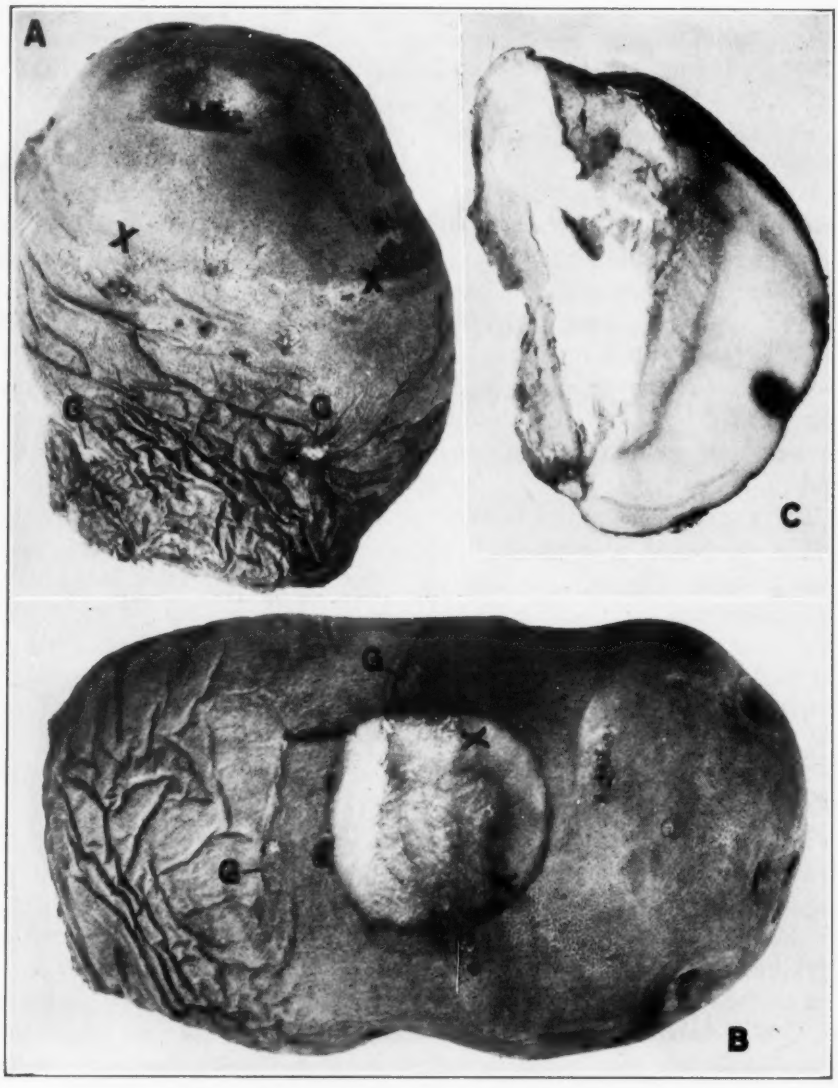

FIG. 1. Green Mountain potatoes. A. Tuber from storage. *Botrytis cinerea* had penetrated through tissues to X–X and emerged (G) from eyes. B. Like A except for cut. C. Half tuber like B except inoculated with pure culture. Reisolation effected from this lesion.

of the same fungus, in due time, tuber infection (Figs. 1, C and 2, C) was obtained identical with that found in storage in 1932, as well as a more shallow, arrested kind of lesion (Fig. 2, B), with most of the surface Pinkish Buff by Ridgway (15, Pl. XXIX) or of color F-4, Pl. 10, in Maerz and Paul (11).

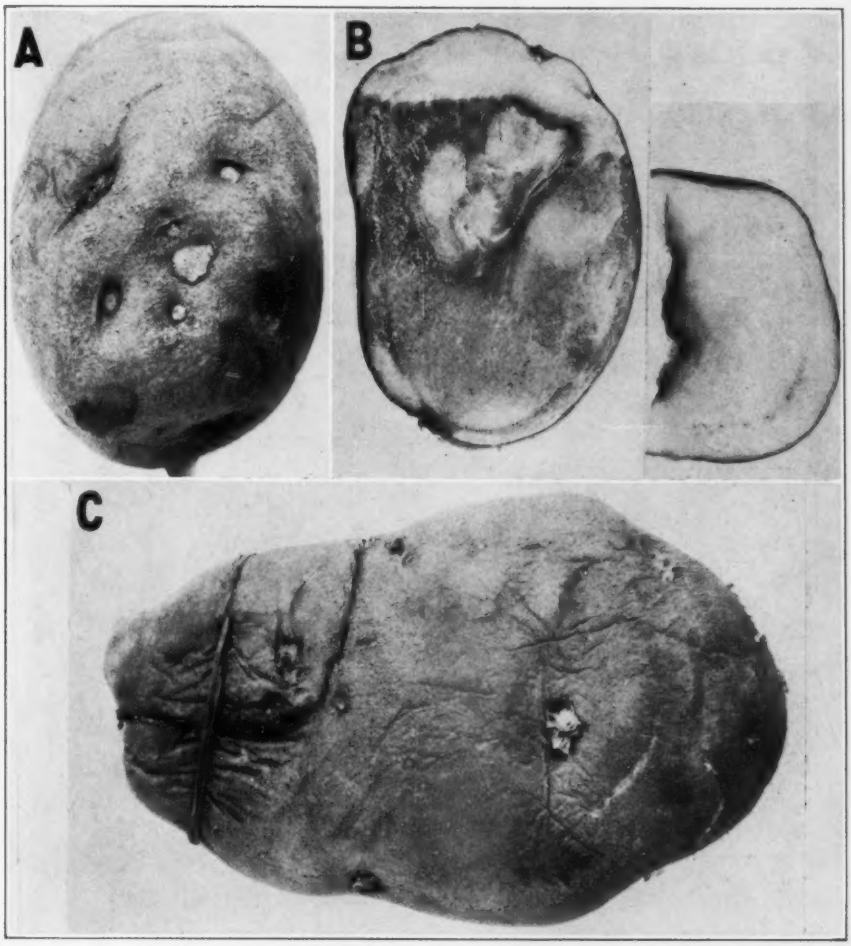

FIG. 2. Green Mountain potatoes. A. Tuber from storage. *Botrytis cinerea* had penetrated through tissues and emerged from eyes. B. Typical infection from pure culture of *Botrytis cinerea*. Left, surface, and right, section, of lesion. C. Like Fig. 1, C, except exterior view. Wrinkled where fungus had penetrated through tissue. Reisolation effected here also.

Cultures of the fungus were kindly examined by H. H. Whetzel, who wrote that it was a Botrytis of the *cinerea* type.

Pethybridge's negative results in infection experiments may have been due to his not having the advantage of certain subsequent studies of the physiology of Botrytis as a pathogen. These studies (2; 3; 4; 5; 6; 7; 8), and others on suberization in potato tubers (1, p. 996; 17, p. 28), induced the writer to use in his inoculations, vigorous young cultures in deep, fresh

TABLE 1.—*Inoculation of potato tubers with Botrytis cinerea*

Series	Month	Inoculated tubers[a]		Check tubers	Storage		Results		
		Age	No.		Temperature	Humidity	Time	Infected[b]	With deep rot
					Degrees C.			*Per cent*	*Per cent*
1	June	Old	35	35	20	Room[c]	In 14 days	60	0
2	July	Old	15	11	20	Room	In 9 days	38	0
		New	12	11	20	Room	In 9 days	25	0
3	August	Old, firm	52	17	5–20	Room or saturated	In 18 days	100	8
3a[d]					20	Room	In 8 weeks	100	17
					20	Saturated	In 8 weeks	100	50
					5	Room	In 8 weeks	100	28
					5	Saturated	In 8 weeks	100	64
4	August	Old, wrinkled	14	0	5	Room or saturated	In 18 days	100	0
							In 8 weeks	100	79
5	October	New	5	5	10	Nearly saturated	In 3 months	60	0
			25[e]	25	10	Nearly saturated	In 3 months	8	0
6	October	New	22	22	10	Nearly saturated	In one month	64	0
			12	12	10	Saturated	In one month	58	17

[a] Of Green Mountain variety except new tubers (Irish Cobbler) of series 2.
[b] No checks infected.
[c] With windows open in a generally rather humid climate.
[d] Same as 3, but at a later date.
[e] Spore inoculations, others being the usual mycelial, both kinds in this series being made in wounds only 1 cm. deep.

wounds, closed tightly after inoculation, and to keep some of the inoculated tubers in a damp chamber at about 5° C. The cultures were nonsporulating ones that had been started with spore suspensions and grown several days on hard potato agar at about 20° C. Usually every second or third tuber in order was used as a check. The checks were treated in the same way as the inoculated tubers except for the absence of any fungus on the inoculating needle. The results of the writer's inoculations are given in the following table.

It is apparent from the table, in inoculation series 3, that deep rot, meaning by this an active penetration by the rot at a depth of 1 cm. or more, was increased somewhat by a lowering of the temperature and was increased distinctly by saturating the air.

In these various inoculations there was no consistent difference between cultures originating from tubers in 1927, from leaves and stems in 1931, or from tubers in 1932. In the sixth series of inoculations, infection was produced similarly by 2 monosporic subcultures and a monohyphal subculture of a culture originating from a tuber in 1927.

For reisolation in series 1, 8 tubers were used, of which one gave a sterile plate and the others *Botrytis cinerea*. Of the latter all were pure cultures except one, which was contaminated with bacteria. These reisolates were used for the majority of the inoculations in series 3 to 6. In series 3 and 4 together, reisolation in 19 days was attempted from only 8 tubers, with positive results in pure culture in all instances. In 2 months, attempted reisolations were successful from 12 of the then tested 21 tubers. Among the remaining 9 failures were 3 that previously had given *B. cinerea,* and 5 of the failures gave as many species of Fusarium. Apparently other fungi sometimes can crowd out *B. cinerea.*

The diseased tubers found by the writer in storage in 1932 appeared to have been infected at the stem end. This, together with the previously quoted observation of Pethybridge on Botrytis tuber rot, indicates that tubers are infected chiefly or only by internal mycelium entering from infected stems through the stolons. This idea is supported further by the comparative failure of spore inoculations in the fifth inoculation series described above. In 1931, conditions favored the Botrytis blighting of potato leaves and stems in Maine so that in some fields this disease caused considerable injury and apparently caused more injury than any other. It seems probable that in the several regions characterized by the occurrence of severe *Botrytis cinerea* blight of potato foliage, namely, Maine,[3] Germany (10), Holland (16, pp. 144–146), Ireland (12; 13; 14, pp. 579–584), and England (9, pp. 42–43), close examination of tubers following such blight will dis-

[3] U. S. Dept. Agr. Bur. Plant Ind., Pl. Dis. Rprtr. **16**: 15. 1932. (Mimeographed.)

close other instances of tuber infection. Such infection may be somewhat masked by late-blight rot and accompanying rots, due to the fact that late blight usually is epidemic in conditions favoring Botrytis blight (14, pp. 579–584).

SUMMARY

Cultures of *Botrytis cinerea* isolated from actively expanding potato-tuber lesions produced the identical disease when inoculated into potato tubers under certain conditions and produced a characteristic shallow lesion under other conditions. Similar results were obtained with cultures of this fungus that had been isolated from soft-rotted tubers several years previously, from lesions on stems and leaves, and from the tubers infected experimentally; and with monosporic and monohyphal subcultures. Mycelial cultures were much more effective than spores in producing infection. From the available data it is concluded that tuber infection probably occurs, following Botrytis blight of the foliage and stems, chiefly or solely as the result of internal mycelium entering from infected stems through the stolons.

MAINE AGRICULTURAL EXPERIMENT STATION,
ORONO, MAINE.

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PHYTOPATHOLOGICAL NOTES

Pecans Infected with Nematospora coryli Pegl.—An organism identified as *Nematospora coryli* Pegl. was isolated from brown areas on kernels of pecan nuts in Florida, in January, 1933. (Fig. 1.) The nuts from which the organism was isolated had been stored from two to three months. In pure cultures on potato-dextrose agar the organism is indistinguishable from similarly-grown cultures of *N. coryli* Pegl. previously isolated[5] from orange, grapefruit, tangerine, satsuma, and tomato in Florida. This is apparently the first time the organism has been isolated from pecans and the only report of it on any variety of nuts except hazelnuts, as reported by Peglion[4] in Italy. It has been reported from various parts of the world by pathologists[1,3,5] on more than 20 other hosts. Investigations

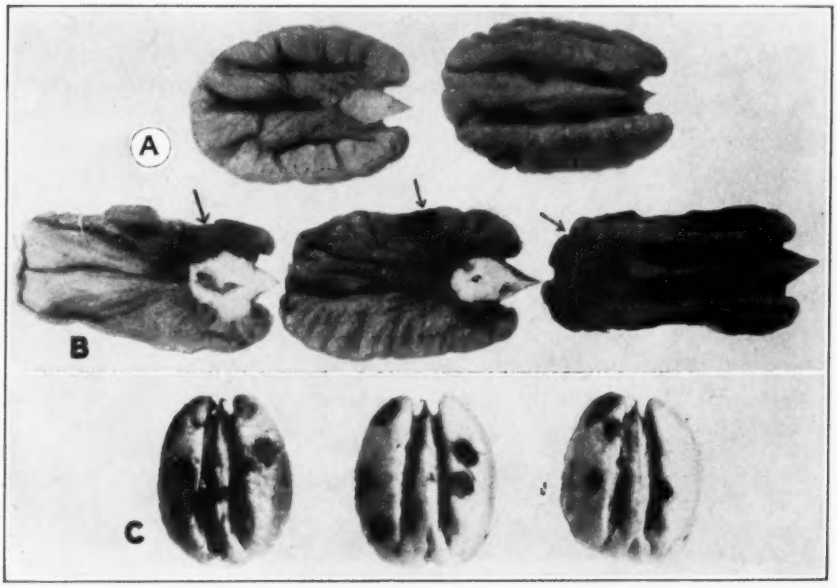

FIG. 1. A. Disease-free half kernels of pecan. B. Half kernels infected with *Nematospora coryli* Pegl. and showing shriveled and darkened tissue. C. "Kernel spot" infections produced by certain sucking insects.

[1] Ashby, S. F., and W. Nowell. The fungi of stigmatomycosis. Ann. Bot. **40**: 69–83. 1926.

[3] Fawcett, H. S., and H. A. Lee. Inspissosis or dry rot of fruit. Pp. 415–420. *In* Citrus diseases and their control. 582 pp. McGraw-Hill Book Company, New York. 1926.

[4] Peglion, Vittorio. Über die *Nematospora coryli* Pegl. Centr. Bakt. Abt. 2, **7**: 754–761. 1901.

[5] Weber, George F. Occurrence and pathogenicity of *Nematospora* spp. in Florida. Phytopath. **23**: 384–388. 1933.

have not been made to determine whether this organism is involved in the shedding of nuts, which is often considerable, during the growing season.

The infection cannot be detected until the shells are removed, when it is found on the convex outer portion of the kernels, usually originating on the tops of the convolutions or on the edges of the half kernels. This is also true to a large extent of the discolored areas known as "kernel spot," which has been shown[2] to be caused by certain sucking insects. The brown color of the diseased area is very similar to the dark brown to black color of kernel spot, and in most instances the two are associated. The areas are slightly sunken and shrunken, showing a visible margin. The tissue below the diseased area is punky and is slightly brownish-stained.

The mode of infection is of primary interest and until experimental data are available, it will be only hypothetical. Insects of the species causing kernel spot have been found feeding on numerous hosts, and *Nematospora coryli* has been isolated from necrotic areas on a score of them. It is very probable that the organism is carried by these sucking insects, resulting in the infection of the pecan kernels.—GEORGE F. WEBER, University of Florida, Gainesville, Florida.

On the Pathogenicity of Wojnowicia graminis.—In Australia, Pittman[1] and others state that *Wojnowicia graminis* (McAlp.) Sacc. causes a root rot of wheat plants. It is also reported as a wheat parasite in Europe by Guyot,[2] Foëx and Rosella,[3] Van de Laar,[4] and others.

While *Wojnowicia graminis* apparently is considered as a parasite on wheat plants in Australia and Europe, it is thought to be a very weak pathogen, at best, in North America. Apart from the statements of McKinney[5] in the United States and Fraser[6] in Canada, that *W. graminis*

[2] Demaree, J. B. Kernel-spot of the pecan and its cause. U. S. Dept. Agr. Bul. 1102. 1922.

[1] Pittman, H. A. Some parasitic and non-parasitic causes of partial or complete emptiness in wheat heads. Dept. Agr. W. Aust. Bul. 301. 1930. (Reprint of: Some parasitic and nonparasitic causes of "empty" or "tipped" heads in wheat. Jour. Dept. Agr. W. Aust. II, 7: 153–164. 1930.)

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[3] Foëx, E., and E. Rosella. Sur les diverses formes du piétin. Rev. Path. Vég. et Ent. Agr. 17: 41–51. 1930.

[4] Van de Laar, J. H. J. Onderzoekingen over *Ophiobolus graminis* Sacc. en *Ophiobolus herpotrichus* (Fr.) Sacc. en over de door deze fungi veroorzaakte ziekten van *Triticum vulgare* Vill. en andere Gramineae. Thesis, Wageningen Agricultural College. 146 pp. 1931.

[5] McKinney, H. H. Foot-rot diseases of wheat in America. U. S. Dept. Agr. Bul. 1347. 1925.

[6] Fraser, W. P. *Wojnowicia graminis.* Rpt. Dominion Botanist (Canada) 1924: 71. 1925.

is only very slightly pathogenic to wheat, there appear to be no very definite experimental data on the subject, except those in Europe reported by Guyot,[2] Foëx and Rosella,[3] and Van de Laar.[4] Therefore, since *W. graminis* is very common in Alberta on the basal parts of both winter and spring wheat, where it frequently is associated with *Ophiobolus graminis* Sacc., it seemed desirable to secure experimental data in this connection.

Single-spore isolations of *Wojnowicia graminis* from pycnidia obtained from wheat plants, growing at 6 widely separated points in Alberta, and 28 isolations of the fungus, obtained from the crowns of wheat plants, taken at random in a field, were tested for virulence on Kharkov, a winter wheat, and Marquis, a spring wheat. The tests were made in the greenhouse in sterilized soil, at a temperature range of 16° to 18° C. Ten grams of inoculum grown on sterilized oat-hull media was placed at seed level in each pot. Thirty wheat plants of each variety were used to test each culture. The plants were examined for disease when 40 days old.

The data secured indicated that none of the 34 isolations of *Wojnowicia graminis* mentioned were significantly pathogenic under the conditions of the experiment. Plants in the sterilized infested soil were as healthy as those grown in sterilized soil. Whether this fungus is pathogenic during the post-seedling stage, under field conditions, must remain an open question until settled experimentally. However, under greenhouse conditions, there was still no apparent evidence of pathogenicity when the plants of the 2 varieties of wheat grew to maturity.

In addition to the pathogenicity tests, measurements were obtained of 100 spores from a pycnidium of *Wojnowicia graminis* on a winter-wheat plant at Cowley, Alberta. The average size was $36.0 \times 3.9\ \mu$, the range being 26.3 to 43.8 μ and 2.5 to 5.6 μ, respectively. The average number of septations was 6.8, with a range of 3 to 8. The data indicate that the spores of *W. graminis* mentioned were slightly larger and wider than those mentioned by Van de Laar, viz., $29.58 \times 3.47\ \mu$, with 5 to 7 septations.—W. C. BROADFOOT, Dominion Laboratory of Plant Pathology at University of Alberta, Edmonton, Canada.

Another Powdery Mildew on Crape Myrtle.—Powdery mildew of crape myrtle has been known in the Gulf States since 1925 when Taubenhaus found it in Texas. In a recent paper[1] the perfect stage of this common crape-myrtle mildew was described as *Erysiphe lagerstroemiae*, n. sp., and the statement was made that no perithecia of any other powdery mildew had been found on this host in the United States. Since that paper was submitted for publication, specimens have been sent to me for identification

[1] West, Erdman. Powdery mildew of crape myrtle caused by *Erysiphe lagerstroemiae*, n. sp. Phytopath. 23: 814–819. 1933.

by Dr. G. W. Carver, of Tuskegee, Alabama, which prove to be *Phyllactinia corylea* (Pers.) Karst. The mycelial growth of these specimens is sparse and patchy, but the few perithecia present are quite typical. However, the general aspect of the mildew caused by Phyllactinia is quite different from that of the common crape-myrtle mildew. In the former only a thin, patchy growth of mycelium and conidia occurs; in the latter there is a heavy and general growth of mycelium and conidia that covers the leaves and stems with a thick, mealy coating. In the numerous collections of powdery mildew on crape myrtle examined in Florida, no perithecia of Phyllactinia have ever been found. Since Phyllactinia is rather common in this State on several other hosts, it seems unlikely that its occurrence on crape myrtle would have passed unnoticed, unless it were rare. Further observations are desirable, especially on the common crape-myrtle mildew, which was reported for years under the name of *Uncinula australiana* McAlp., either with or without an interrogation point.—ERDMAN WEST, Florida Agricultural Experiment Station, Gainesville, Florida.

BOOK REVIEWS

Rawlins, Thomas Ellsworth. *Phytopathological and Botanical Research Methods.* ix + 156 pp., 3 figs. John Wiley & Sons, Inc., New York. 1933. Price $2.50.

Methods of research and technique of plant pathology, botany, and other biological sciences are published in a large number of books, journals of all kinds, and often in obscure periodicals not generally read. Investigators, therefore, will welcome any attempt to bring together in a single volume in an orderly arrangement the methods that have been found useful in research work. The volume bearing the above title is an attempt in this direction. The author has not only drawn generously from the publications of research workers, but he has made liberal use of methods developed by himself as a teacher and investigator. The book is divided into three parts as follows: Part I. Choosing and Planning a Research Project; Part II. Experimental Methods; Part III. Interpretation of Experimental Results.

Parts I and III are both short and probably contribute least to the value of the book. It is not easy to tell an investigator how to choose a project for work, if indeed he has any choice in the matter, or how to interpret the results after they are obtained. Part II, on the other hand, includes a mass of data and serves as a source of information on methods and technique. The book spares the investigator either the necessity of memorizing the various methods to be applied in any particular line of work or that of searching for them in the published works of others.

The author has probably made his greatest contribution in the chapter entitled "Microscopic Methods," and especially that part under microchemistry. Here he has collected in a concise form methods that may be employed by the investigator as type material in his comparative work. His statements and discussions are supported by citations of the work of other investigators in a similar field. As complete as the book is, some subjects regarded by the reviewer as important, unfortunately, have been omitted or only inadequately treated. For example, under culture media methods especially applicable for use in growing cultures to be used in soil inoculations are entirely disregarded. Again, under sterilization methods nothing is said about soil sterilization that is likely to be more difficult than the mastery of the autoclave to which he gives a paragraph. With the increasing interest and importance of diseases caused by soil-inhabiting organisms, a knowledge of soil sterilization and soil inoculation and management is especially important, yet this phase of the subject is sparingly discussed. The very important subject of inoculating plants is given less than two pages.

This phase of phytopathology is highly important and more space could profitably have been given to a fuller discussion of methods and technique.

The failure to obtain infection is often due not to a lack of parasitism of a particular organism but to a lack of the proper relation of the host with respect to temperature and humidity. Temperature and water content of the soil also are governing factors in the infection by soil organisms. The importance of air temperature and moisture and temperature and water content of the soil in relation to infection and methods for their control might very profitably have been treated at some length.

The book closes with 960 citations and an excellent index. There is no question of the amount of work involved in working through such a large number of articles and extracting therefrom methods that contribute to phytopathological science. The citations are well chosen and themselves classified under—1, Histology and Cytology; 2, Microchemistry; 3, Cultural Methods; 4, Viruses; 5, Miscellaneous Methods; 6, Interpretation of Experimental Results.

On the whole the book is excellent and deserves, for ready reference, a place on the shelf of all phytopathologists and others engaged in botanical research.—L. L. HARTER, Washington, D. C.

Raber, Oran. *Principles of Plant Physiology.* vii + 432 pp., 29 figs. Edition II Revised, Macmillan Co. New York. 1933. Price $3.50.

This is a revised and enlarged edition of the excellent elementary text, which was first published in 1928. The number of chapters has been increased from 31 to 33 and the number of pages from 377 to 427. The chapter headings remain very much the same as they were in the first edition, except the chapters on Growth and Reproduction. The former has been divided into two chapters, "General Characteristics of Growth" and "Specific Growth Factors" and the latter into two chapters "Reproduction" and "Reproductiveness and Seed Germination." New sections have been added on hydrogen-ion concentration and photoperiodism, while rather extensive additions have been made to other topics in discussing them more intensively or in bringing them up to date.

It is always a question to the teacher of any subject how much of related matters should be included in the presentation of the material in his special field. It is thus a moot question how much of subjects that are vehicles of expressing physiological facts should be discussed in a text on plant physiology. It is doubtful if colloids and hydrogen-ion concentration can be so adequately expressed as to be of much benefit to a student in a page or two of an elementary text on plant physiology. A student who elects or is required to take elementary physiology should have been exposed sufficiently

to these topics in a general way in elementary chemistry so that little or no mention of them should be necessary.

To the reviewer, it seems that, in the discussion of the cell in Chapter II, too much emphasis and space are devoted to the subject of colloids and too little to the morphological entities of the cell. The relationships of colloidal behavior to the activities of the cell have been much over-emphasized by plant physiologists, for, in the last analysis, our knowledge of these relationships is very meager indeed.

The references to literature made in the text simply by citing the author's name and the date, without their being listed elsewhere in the book, are very confusing to those who desire to inform themselves further on the topic under consideration. Few indeed, if any, are the teachers who can recall from their knowledge of the literature the significance or importance of these citations by such abridged references. A footnote simply stating the volume and page of the reference would be an invaluable aid to the teacher as well as to the student.

The adverse criticisms that have been offered should, however, be given only minor consideration in view of the excellent presentation in an elementary way of the various topics of plant physiology. The writer knows from experience that it is much easier for a reviewer to criticize a text adversely than it is to write a text that is above criticism.

The author shows the aptitude of the teacher by missing no opportunity to cite the relationships of his subject to human affairs. This is one of the keys that always unlocks the door to the interest of the student. The list of thought-provocative questions at the end of each chapter is well chosen and will further incur the interest of the student in the subject.

The text throughout is written in a clear and concise manner and in a simple style that is easy for a student to follow. The context is well-balanced and no undue emphasis is placed on any topic, while the whole field of plant physiology is brought before the reader.

The advanced student in plant physiology would do well to read the text, for, by so doing, he would obtain a well-presented summary of that field.

The book in its content and in the manner of its presentation is a valuable contribution to the literature of elementary plant physiology.—EDWIN C. MILLER, Kansas State College, Manhattan, Kansas.

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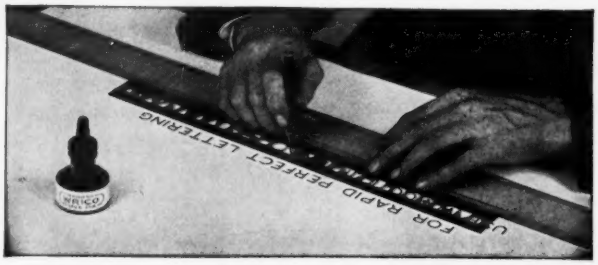

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